A Thesis submitted for the degree of

Doctor of Philosophy

# Substance-specific modulation of the affective and neurobiological effects of heroin and cocaine in human addicts

Silvana De Pirro

School of Psychology


University of Sussex

August 2017

# Declaration

I hereby declare that this thesis has not been and will not be submitted, in whole or in part, to another University for the award of any other degree.

Signature:

Name: Silvana De Pirro

# Summary

This dissertation investigates how the settings of drug use influence the affective and neurobiological response to heroin versus cocaine in addicts.

Chapter 1 reviews the neuropharmacology of heroin and cocaine and the theoretical background for drugs-settings interactions, including a detailed discussion of findings from previous studies in animals and humans that show how the same settings can influence in opposite directions the reinforcing effect of heroin and cocaine. Cocaine self-administration, for example, was greatly facilitated when rats were tested outside the home environment relative to rats test at home. The opposite pattern was found for heroin. Translational studies in humans yielded similar results. Indeed, heroin and cocaine co-abusers reported using the two drugs in distinct settings: heroin preferentially at home and cocaine preferentially outside the home. The aim of this dissertation is to determine whether the setting could also influence in opposite manner the affective and neurobiological response to heroin and cocaine in human addicts.

Chapter 2 illustrates the findings of a study aimed at testing the hypothesis that the affective state experienced under cocaine or heroin is the result of an interaction between central and peripheral drug effects and the surroundings of drug use. According to this hypothesis, when cocaine is taken at home there is a mismatch between the familiar environment and the peripheral effects such as arousal, increased heart rate, increased respiratory rate, and increased muscular tension (which are usually produced in stressful situations). This mismatch dampens cocaine-rewarding effects. A mismatch would also occurs when heroin (which produces sedation and decreases heart rate, respiratory rate, and muscular tension) is used outside the home in contexts requiring vigilance. We found indeed that co-abusers subjectively experienced opposite changes in arousal, heart rate, respiratory rate, and muscular tension in response to cocaine (increase) versus heroin (decrease). Most important, using a novel two-dimensional visual test, we found that in agreement with the working hypothesis the valence of the affective state produced by heroin and cocaine shifted in opposite directions as a function of the setting of drug use: heroin was reported to be more pleasant at home than outside the home, and vice versa for cocaine.

Chapter 3 illustrates the results of in which emotional imagery was combined with fMRI to investigation the neurobiological underpinnings of drug and setting interactions in

addicts. Heroin and cocaine co-abusers were asked to recreate real-world settings of drug use during fMRI. In agreement with the working hypothesis, we found that heroin and cocaine imagery produced opposite changes in BOLD in the prefrontal cortex and in the striatum, regions implicated in brain reward in humans. Furthermore the same pattern of dissociation was observed in the cerebellum, suggesting that that a fronto-striatal-cerebellar network is implicated in processing drug-setting interactions.

Chapter 4 includes a summary of the results, a general discussion, and suggestions for future research and implication. The major finding is that the environment surrounding drug use can influence in opposite manner the affective and neurobiological response to heroin and cocaine, suggesting that therapeutic approaches to the treatment of drug addiction should take into account the distinctive effects of different classes of drugs as well as the contexts of drug use.

The Appendix includes reprints of two papers reporting on additional studies conducted during the course of the Ph.D. program, which are not directly germane to the aims of the dissertation. Other three papers are in the pre-submission stage.

# Table of Contents

# List of Figures

## Chapter 1



## Chapter 2

## Chapter 3

# List of Tables

# List of Abbreviations

| | |
|---|---|
| 2-D/3-D | Two-Dimensional/ Tri-dimensional |
| 5-HT | Serotonin |
| 6-MAM | 6-Monoacetylmorphine |
| ANS | Autonomic Nervous System |
| BA | Broadman Area |
| BOLD | Blood Oxygenation Level Dependent |
| cAMP | cyclic Adenosine Monophospate |
| CNS | Central Nervous System |
| CCAS | Cerebellar Cognitive Syndrome |
| COMT | Catechol-O-Methyl Transferase |
| CS | Conditioned stimulus |
| DA | Dopamine |
| DAT | Dopamine Transporter |
| DOR | Delta Opioid Receptors |
| EMG | Electromyography |
| EPI | Echo-Planner Imaging |
| FC | Functional Connectivity |
| FIR | Finite Impulse Response |
| FWE | Family-Wise correction |
| fMRI | functional Magnetic Resonance Imaging |
| FOV | Field of View |
| FWHM | Full Width at Half Maximum |
| GLM | General Linear Model |
| HRF | Hemodynamic Response Function |
| *Ki* | Inhibitory constant |
| KOR | Kappa Opioid Receptors |
| M3G | Morphine-3-Glucoronide |
| M6G | Morphine-6-Glucoronide |
| MEG | Magnetoencephalography |
| MNI | Montreal Neurological Institute |
| MOR | Mu Opioid Receptors |
| MPRAGE | Magnetization-Prepared Rapid Gradient-Echo |

| | |
|---|---|
| MRI | Magnetic Resonance Imaging |
| MSN | Medium Spiny Neuron |
| NAcc | Nucleus Accumbens |
| NE | Norepinephrine |
| NET | Norepinephrine Transporter |
| PMAT | Plasma Membrane Monoamine Transporter |
| PFCx | Prefrontal Cortex |
| PET | Positron Emission Tomography |
| PNS | Peripheral Nervous System |
| ReML | Restricted Maximum Likelihood |
| rCBF | regional Cerebral Blood Flow |
| ROI | Region of Interest |
| rs-fMRI | resting state fMRI |
| RSN | Resting State Network |
| SC | Skin Conductance |
| SD | Standard Deviation of the Mean |
| SN | Substantia Nigra |
| SEM | Standard Error of the Mean |
| SERT | Serotonin Transporter |
| SMA | Supplementary Motor Area |
| SNS | Sympathetic nervous system |
| SPM | Statistical Parametric Mapping |
| TE | Echo Time |
| TR | Repetition Time |
| UCS | Unconditioned Stimulus |
| USV | Ultrasonic Vocalization |
| VAS | Visual Analogue Scale |
| VTA | Ventral Tegmental Area |

# Acknowledgments

My grateful thanks to all the patients of the Addiction Clinic Villa Maraini, for their participation and commitment in the projects presented in this dissertation.

Many thanks to my doctoral supervisor Professor Aldo Badiani, endless source of scientific knowledge, patience, and support, who gave me the freedom to develop my own research interest and respected my 'obsession with methods', but at the same time was indefinitely generous with his valuable advice and guidance whenever it was needed.

Many thanks also to my co-supervisor Professor Theodora Duka for her kind encouragement during the past few years.

I am extremely grateful to my advisor Dr. Hans Crombag for the enriching scientific interactions and his constant and invaluable support, especially during this last period.

The studies reported here would not have been possible without Giovanni Iavarone, a social worker at Villa Maraini and the best collaborator that I ever had, for his supportive, encouraging and professionals attitude during the fMRI study. Giò had an incredible work ethic and he showed me what is possible to overcome and achieve with a co-ordinated and efficient teamwork. He was my first source of knowledge when I started to work with drug addicts and he provided me with a safe environment all the time. He was a precious friend and he still missed so much.

I wish to thank Dr. Ettore Rossi and all the social workers of the Addiction Clinic Villa Maraini in Rome, who provided the facilities and the support necessary for the completion of the studies presented in this dissertation.

Many thanks to Professor Luigi Pizzamiglio, towering figure in the field of Neuropsychology, and Dr. Gaspare Galati for giving me the possibility to work at the Brain Imaging facilities of the Santa Lucia Foundation. A special thank to Dr. Maddalena Boccia, Steve Gazzitano, and the radiographers of the Santa Lucia Foundation, who provided excellent technical support.

Many thanks to Professor Jenny Rusted, for her constant friendship during my Ph.D. and for made me move back on the tennis courts after years.

I am most grateful to Professor Mark Jenkinson and to all the tutors of the Graduate Programme of the FMRIB at Oxford University, where I received the best methodological training in brain imaging techniques I could have ever wished for. This last year gave me the opportunity of being exposed to an amazing interdisciplinary community of inspiring and leading scientists in the neuroimaging field and I deeply treasure each moment spent at FMRIB.

My grateful thanks also to my precious "non-academic-gym-related" net of support: first of all Chris (who, during the past three years, listened, supported and encouraged me far beyond what my words could ever express), and then Emily, Henry, Victoria, and Seda, an

invaluable team of amazing, creative, busy and wholehearted women, who constantly inspired me.

I wish to find the words to thank Marco for always being there for me. Despite all, he has been a constant source of support and security and he always will be.

Finally, I dedicate this dissertation to the memory of my beloved grandparents Francesca and Orazio, and to my Mum, without whom I would never have had the opportunity to receive a higher education and to pursue my passion for brain research.
My greatest thanks to them ... with all my love.

# Chapter 1

## Theoretical and Experimental Background

### 1. Introduction

It is often thought, by experts and laypersons alike, that addictive drugs are fundamentally the same. The 2016 Surgeon General's Report on Alcohol, Drugs, and Health, for example, offered a very simplified picture of drug addiction, according to which all drugs produce subjective pleasure via common mechanisms of action and, when taken repeatedly, alter the brain in a similar manner, resulting in withdrawal symptoms after abstinence, compulsive craving, and eventually relapse into drug seeking. In the official website the National Institute for Drug Abuse (https://www.drugabuse.gov/publications/media-guide/science-drug-abuse-addiction-basics, accessed August 4, 2017) it is stated: "Nearly all addictive drugs directly or indirectly target the brain's reward system by flooding the circuit with dopamine. Dopamine is a neurotransmitter present in regions of the brain that regulate movement, emotion, cognition, motivation, and feelings of pleasure. The overstimulation of this system, which rewards our natural behaviors, produces the euphoric effects sought by people who use drugs and teaches them to repeat the behaviour."

This tendency to oversimplification is not unique to policy documents or institutional websites, which target a non-academic audience. Most theoretical

frameworks of drug reward[1] and drug addiction are based on core shared processes and mechanisms, even if these differ greatly from theory to theory. Of course, for some drugs the similarities in mechanisms of action are undisputed. Opioid drugs such as heroin, morphine, and fentanyl, for example, are all agonists to the mu opioid receptors; psychostimulant drugs such as cocaine, amphetamine, and methamphetamine share the ability to bind the transporter responsible for the reuptake of dopamine into the synaptic terminal (leading to the blockade of dopamine reuptake in the case of cocaine and to the reverse transport of dopamine in the case of amphetamines); alcohol, benzodiazepines, and barbiturates bind different sites on the $GABA_A$ receptors. However, as discussed in the next sections, it is generally held that the neurobiological cascades of all drugs converge at a distal level on a common transmitter system: the mesolimbic dopaminergic system, which is responsible for mediating the core rewarding effect of all addictive drugs. Furthermore, shared neuroplastic adaptations in the dopaminergic system and related circuitry are thought to underlie the transition to drug addiction after prolonged exposure to addictive drugs.

Yet, research conducted in the past three decades has provided experimental evidence of major differences among the various classes of addictive drugs (for reviews see Badiani et al. 2011; Peters et al. 2013; Nutt et al. 2015). Actually, it is fair to say that the few studies that investigated more than one class of addictive drugs have repeatedly shown the reinforcing effects of these drugs are not affected in the same way by pharmacological or

[1] The term *reward* is used in different ways in empirical and theoretical papers. In many cases, reward is used as a synonym of reinforcement, and rewarding effect as a synonym of reinforcing effect. For the purpose of this dissertation the term reward is used to indicate the hypothetical psychological construct that is responsible for the reinforcing effects of certain stimuli.

neurobiological manipulations and, when administered chronically or intermittently, do not produce the same type of neuroplastic adaptations.

Of course, it is reasonable to assume that both shared and unique mechanisms and processes are implicated to different extent in the rewarding and addictive properties of addictive drugs (and even in those of other non-pharmacological rewarding stimuli, such as food, sex, gambling, etc.). The challenge for the field is to understand whether the differences among the various classes of drugs are only a minor detail in the great picture or if they should be taken into account for an understanding of the neurobiological bases of drug reward and drug addiction and for the development of effective treatments.

The aim of the present dissertation was to contribute to this inquiry by exploring the differential role of the setting of drug use in modulating the rewarding effects of two prototypical addictive drugs, heroin and cocaine in human addicts. Previous studies have shown in fact that the setting of drug taking can exert a substance-specific influence on the response to these two drugs as well as to other drugs of abuse (Badiani 2013).

In Section 2 below, I will review basic information concerning the pharmacodynamics and pharmacokinetics of heroin and cocaine. In Section 3, I will discuss the neurobiological bases of drug reward and examine the overlap in the more distal mechanism of action of heroin and cocaine. In Section 4, I will discuss the role of environmental factors in drug addiction and in particular I will review the findings from a series of studies providing the background for the present dissertation. Finally, in Section 5, I will provide an outline of the two studies included in the dissertation.

Chapter 2 will be devoted to the first study, which was concerned with the role of setting in modulating the affective state induced by heroin versus cocaine in human addicts who co-abused both drugs. The major finding from this study is that the affective states induced by heroin and cocaine are very different and are influenced by the setting of drug use in a substance-specific manner.

Chapter 3 will report on the second study, which coupled emotional imagery evoking heroin and cocaine use in different settings with fMRI scanning to investigate the neural correlates of drug-setting interactions. I found that the pattern of brain activation during drug imagery was the result of a complex interaction between drug and setting.

Finally, Chapter 4 will provide a general discussion of the findings of my dissertation.

## 2. Primary mechanisms of action of heroin and cocaine

### 2.1. Pharmacology of heroin

Heroin (diacetylmorphine) binds the mu opioid receptors (MOR) and with lesser affinity the delta opioid and the kappa opioid receptors (DOR and KOR, respectively). The MOR, DOR, and KOR are seven-transmembrane domain receptors coupled with a $G\alpha_{i/o}$ protein (Figure 1, left panel). The transductional cascade of opioid receptors is thought to be mostly 'inhibitory' of cell functions in that their activation inhibits the synthesis of cAMP, via inhibition of adenylyl cyclase by the $\alpha_i$ subunit, and hyperpolarizes the neuron,

via the βγ complex-induced activation of inwardly rectifying potassium channels (Stein 2016). A second transductional pathway is represented by the β–arrestins (Figure 1, right panel), which are involved in receptor internalization, and are thought to be implicated in respiratory depression and constipation, as well as in the development of opioid tolerance (Manglik et al. 2016).

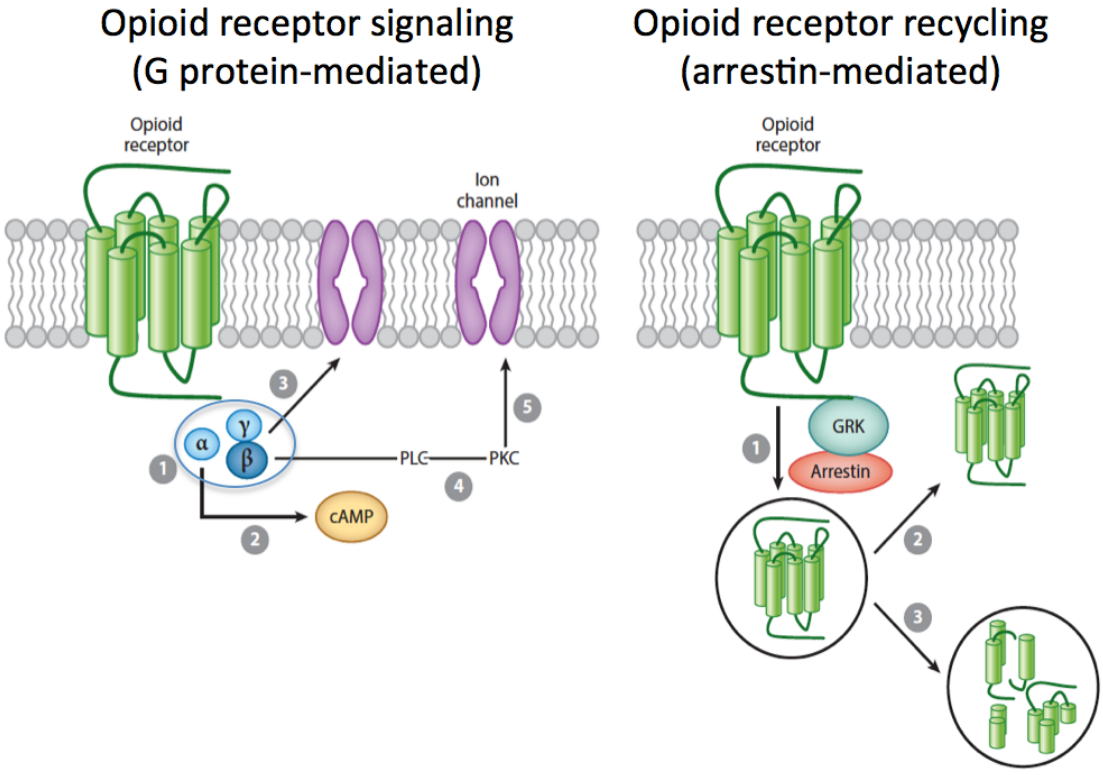



**Figure 1. Left panel: opioid receptor signaling.** Opioid agonists such as heroin, 6-MAM, morphine, and M3G bind the mu opioid receptors, thereby inducing the dissociation of G protein into the Gαi and Gβγ subunits. The Gαi subunit inhibits the adenylyl cyclase thus reducing cAMP (2). The Gβγ subunits close voltage-gated Ca2+ channels and open rectifying K+ channels (3). The Gβγ subunits can also activate the phospholipase C/phosphokinase C pathway (4), which in turn modulates Ca2+ channels (5). **Right panel: opioid receptor recycling.** Opioid agonists also activate the G protein–coupled receptor kinase (GRK), which in turn phosphorylates the activated receptor. Phosphorylated receptors can be bound by arrestins thus undergoing desensitization (1) and internalization via a clathrin-dependent pathway. After internalization the receptors are recycled to the cell membrane (2) or degraded within the lysosomes (3). Modified from Stein 2016.

Opioid receptors are widely distributed throughout the peripheral and central nervous systems (PNS and CNS, respectively), and therefore their

activation produces a host of physiological and behavioural responses. As discussed in Section 3, the rewarding effects of heroin are thought to depend mostly on the activation of MOR located in the mesocorticolimbic circuitry of the brain.

The estimated plasma half-life of heroin in the human body is less than 5 minutes (Rook et al. 2006). After entering the blood, heroin is in fact rapidly transformed by esterases into 6-monoacetylmorphine (6-MAM), which is further de-acetylated to morphine (Inturrisi et al. 1984; Rentsch et al. 2001). Both 6-MAM and morphine are potent MOR agonists in their own right. Morphine is further metabolized to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) (Milne et al. 1996). While M3G has relatively little biological activity, M6G is a potent agonist at MORs (Ulens et al. 2001; Penson et al. 2000; Christrup 1997). Morphine has a terminal half-life of 2-3 hours in healthy subjects (Wagner and O'Hara 1997). Thus, it is commonly held that the effects of heroin are mostly mediated by its metabolites, above all by morphine (Gutstein and Akil 2006).

Yet, there is some evidence against the notion that heroin is simply a pro-drug of morphine. In the first place, it has long been noted that heroin has greater euphorigenic effects than morphine, a phenomenon that has been attributed to the greater lipophilicity of heroin relative to its metabolites, resulting in a more rapid onset of action (Eddy et al. 1957; Martin and Fraser 1961). Furthermore, Pasternak and colleagues have proposed the existence of MOR subtypes and in particular of a MOR-1 splice variant that has high affinity for heroin and M6G but little affinity for morphine (Rossi et al. 1996; Brown et al. 1997). This is an intriguing phenomenon given that plasma and urine of heroin addicts have been reported to contain more M6G and less M3G than

those of heroin-naive individuals treated with morphine for pain control (Antonilli et al. 2003) and that prolonged exposure to high doses of morphine does not appear to influence M3G or M6G synthesis (Faura et al. 1998).

**2.2. Pharmacology of cocaine**

Cocaine is a drug with at least two distinct mechanisms of action. First, it blocks the voltage-gated Na+ channels located in the non-myelinated portions of axons, thereby blocking the origination and transmission of action potentials. This mechanism is responsible for the well-known local anaesthetic effects of cocaine. The second mechanism of action is represented by the interaction with the specialized transporter proteins that mediate the recapture of monoamines from the extracellular space back into the cytoplasm (Amara and Kuhar 1993; Masson et al. 1999). These monoamine transporter proteins belong to a superfamily of $Na^+/Cl^-$ dependent transporters that share genetic, structural, and functional homologies (Blakely et al. 1994; Uhl and Johnson 1994). There are distinct transporters responsible for the reuptake of the different monoamines: i) dopamine (DA) neurons express dopamine transporters (DATs), ii) epinephrine and norepinephrine (NE) neurons express NE transporters (NETs), and iii) serotonin (5-HT) neurons express high affinity 5-HT transporters (SERTs) and low affinity plasma membrane monoamine transporters (PMATs) (Rothman et al. 2003; Zhou et al. 2007).

Under physiological conditions, the reuptake represents the principal mechanism for the termination of monoaminergic signaling at a synaptic level. However, in the case of dopamine (see Figure 2) and the other two

catecholamines, there is an additional mechanism of signal termination represented by enzymatic degradation to 3-methoxytyramine by the catechol-O-methyl transferase (COMT).

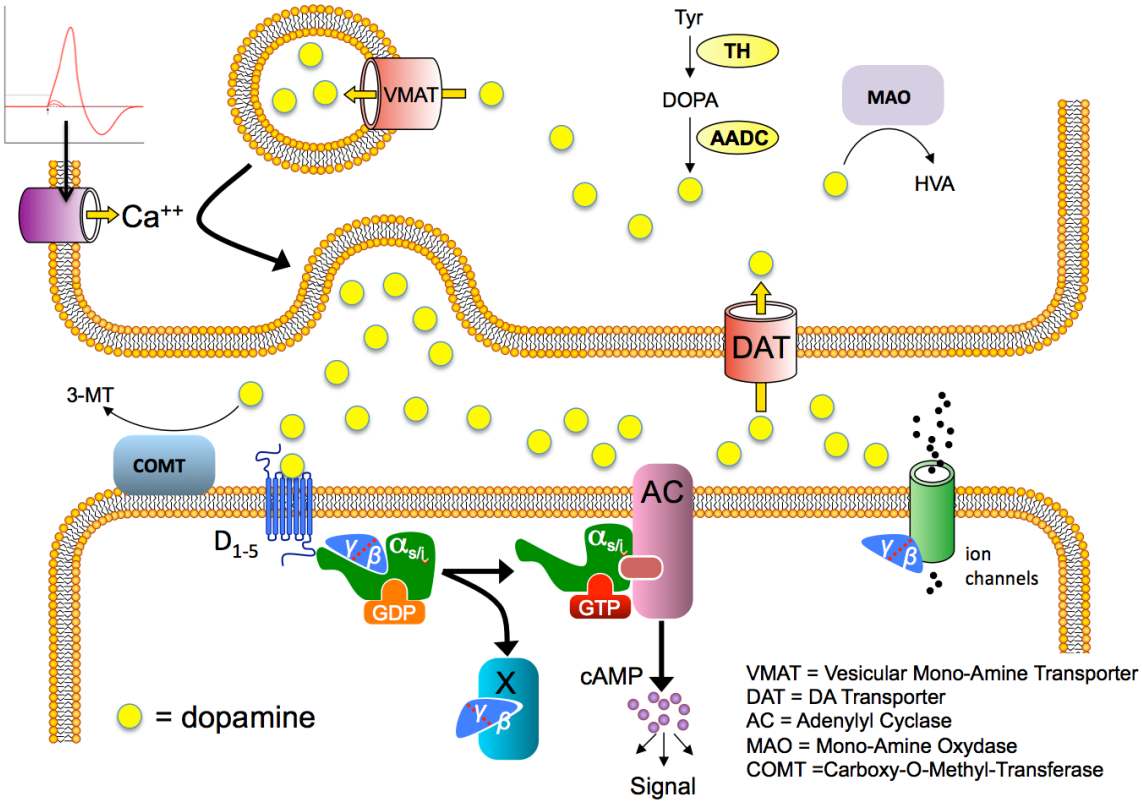



**Figure 2.** Dopaminergic synapse.

The relative role of reuptake versus enzymatic degradation in terminating catecholamine transmission depends on the type of neuronal firing and on the density of the transporter. In the case of the meso-telencephalic dopaminergic neurons, for example, COMT is thought to play the major role in terminating dopamine tonic transmission in the nucleus accumbens (NAcc) as well as phasic transmission in the prefrontal cortex (PFCx). The DAT is thought instead to play a major role for the termination of phasic transmission in the NAcc (see Figure 3).

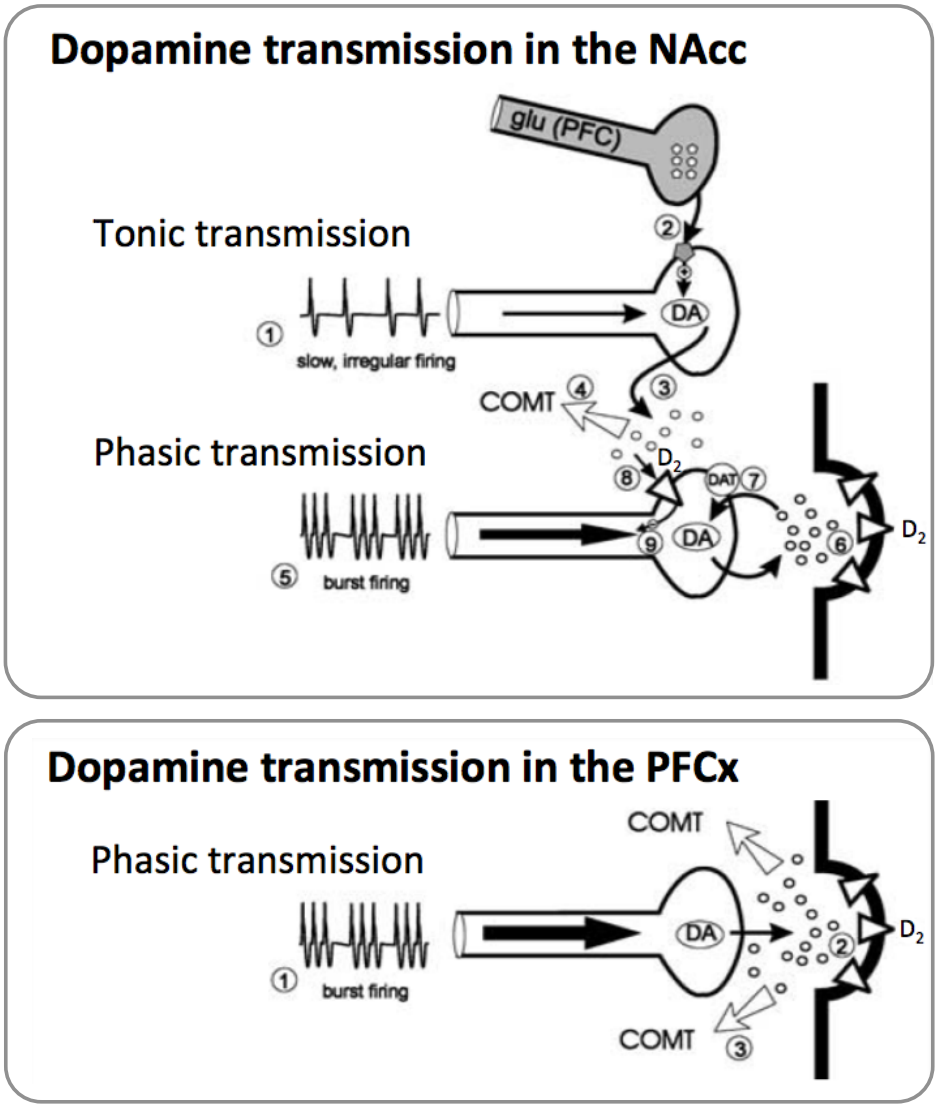



**Figure 3. Top panel: dopamine transmission in the nucleus accumbens (NAcc).** Tonic dopamine (DA) release is dependent on slow, irregular spike activity of meso-accumbens dopaminergic neurons (1) and is thought to be modulated by glutamatergic afferents from the PFCx and other brain regions (2). Slow, irregular firing produces low of dopamine concentrations (5–20 nM) in the extrasynaptic space (3), where dopamine is degraded by COMT (4). Phasic dopamine transmission is triggered by burst firing of meso-accumbens dopaminergic neurons (5), which produces high dopamine concentrations (in the mM range) in the synaptic cleft, where it stimulates postsynaptic D2 receptors (6). Synaptically released DA is transported back into the synaptic terminal by the DAT (7). The concentrations of tonically released dopamine are too low to stimulate low-affinity postsynaptic D2 receptors, but are sufficient to stimulate high-affinity presynaptic D2 receptors (8), thus inhibiting phasic dopamine release (9). **Bottom panel: dopamine transmission in the prefrontal cortex (PFCx).** Meso-cortical dopamine neurons fire only phasically (1), thus producing high concentrations of dopamine into the synaptic cleft (2). Dopaminergic terminals in the PFCx do not contain high levels of DAT. Thus, the termination of dopamine transmission in the PFCx depends mainly on degradation by COMT. Modified from Bilder et al. 2004.

Cocaine binds the transporters at the same binding site of endogenous monoamines (Beuming et al. 2008). However, after binding to the transporters cocaine is not transported inside the neuron but blocks the reuptake of monoamines, thereby elevating their extracellular concentration. Thus, cocaine acts as an indirect agonist at the receptors for dopamine ($D_{1\text{-}5}$), NE ($\alpha_{1\text{-}2}$ and and $\beta_{1\text{-}3}$), and 5-HT ($5\text{-}HT_{1A,\ 1B,\ 1D,\ 1E,\ 1F,\ 2A,\ 2B,\ 2C,\ 3,\ 4,\ 5A,\ 5B,\ 6,\ 7}$). The above-mentioned receptors are all seven-transmembrane domain G protein-coupled receptors, with the exception of the $5\text{-}HT_3$ subtype, which is a ligand-gated $Na^+/Cl^-$ channel.

Cocaine does not exhibit much selectivity towards the different monoamine transporters. Uptake inhibition assays have shown in fact comparable $K_i$s for DA, NE, and 5-HT reuptake (Rothman et al. 2003). Given that DA, NE, and 5-HT neurons are widely distributed throughout the PNS and the CNS, it is not surprising that cocaine administration results in a host of diverse physiological and behavioural responses. However, the rewarding effects of cocaine are thought to depend mostly on its actions on the terminals of the meso-accumbens dopaminergic neurons (see Section 3). The postsynaptic effects of dopamine differ as a function of receptor subtypes. The activation of $D_1$ and $D_5$ receptors ($D_1$-like receptor family) results in increased cAMP levels (via activation of adenylyl cyclase by a $G\alpha_{s/olf}$ protein). In contrast, activation of $D_{2\text{-}4}$ receptors ($D_2$-like receptor family) decreases cAMP levels (via inhibition of adenylyl cyclase by a $G\alpha_{i/o}$ protein) and decreases excitability via $\beta\gamma$ complex-induced activation of inwardly rectifying potassium

channels. The pharmacology of dopamine receptors is very complex, also because they can undergo autodimerization as well as heterodimerization with each other (e.g., $D_1$-$D_2$, $D_1$-$D_3$, $D_2$-$D_4$) or with other receptors (e.g., with adrenergic and adenosinergic receptors) (Beaulieu et al. 2015). Dopamine $D_2$-like receptors have greater affinity for dopamine than $D_1$-like receptors. While $D_2$-like receptors are located both at a presynaptic (auto-receptors) and postsynaptic level, $D_1$-like receptors are located only at a post-synaptic level (see Figures 2 and 3).

After intravenous administration in humans, the half-life of cocaine is about 40-80 min (Jones 1990; Sholar et al. 1998; Perez-Reyes et al. 1994; Jeffcoat et al. 1989). Less than 5% of cocaine is excreted as such in the urine. Most of it is metabolized in the liver by hydrolytic ester cleavage. The main metabolites of cocaine (all inactive) are represented by benzoylecgonine, ecgonine methyl ester, and ecgonine. When cocaine is co-abused with alcohol, however, the ethyl ester of benzoylecgonine (cocaethylene) is formed (Jatlow 1991). The $K_i$ for dopamine reuptake of cocaethylene is comparable to that of cocaine, whereas the $K_i$ for NE and 5-HT reuptake is much higher, indicating greater selectivity of cocaethylene for DAT relative to NET and SERT (Jatlow 1991; Rothman et al. 2003). Cocaethylene has been found to be equipotent to cocaine with regard to self-administration in primates and rats (Jatlow 1991; Landry 1992) and although it appears to be slightly less euphorigenic than cocaine in humans (Perez-Reyes et al. 1994) it is quite possible that it significantly contributes to the combined rewarding effects of cocaine and alcohol.

## 3. Neurobiology of cocaine and heroin reward

### 3.1. Neural substrates of reward

It is beyond the scope of this dissertation to provide an in-depth discussion of the neurobiological bases of reward. However, it is important to point out that research conducted in the past five decades has identified a number of brain areas that participate in processing the hedonic properties and the incentive motivational value (incentive salience) of rewarding stimuli (and, via associative learning, of conditioned stimuli), and their modulation by cognitive and emotional processes, as well as by homeostatic signals.

Figure 4 illustrates some of the circuits that are thought to play a major role in reward processing (Russo and Nestler 2013). As discussed below, the greatest degree of attention has focused on the role of the dopamine neurons originating in the ventral tegmental area (VTA) and substantia nigra (SN) and projecting to the NAcc (ventral striatum), caudate-putamen (dorsal striatum), prefrontal cortex (PFCx), amygdala, and hippocampus. In turn, prefrontal cortex, hippocampus, and amygdala send excitatory glutamatergic projections to the NAcc/striatum and to the VTA/SN. As discussed below, the NAcc/striatum exert a complex control on the VTA/SN (which in addition to dopaminergic neurons contains GABAergic interneurons and GABAergic neurons projecting to the thalamus) through two separate pathways.

Based on a massive volume of theoretical and experimental work, it is possible to assign different functional roles to the glutamatergic inputs to the NAcc, even though there is no agreement about the fine details (Russo and Nestler 2013; Richard et al. 2013).

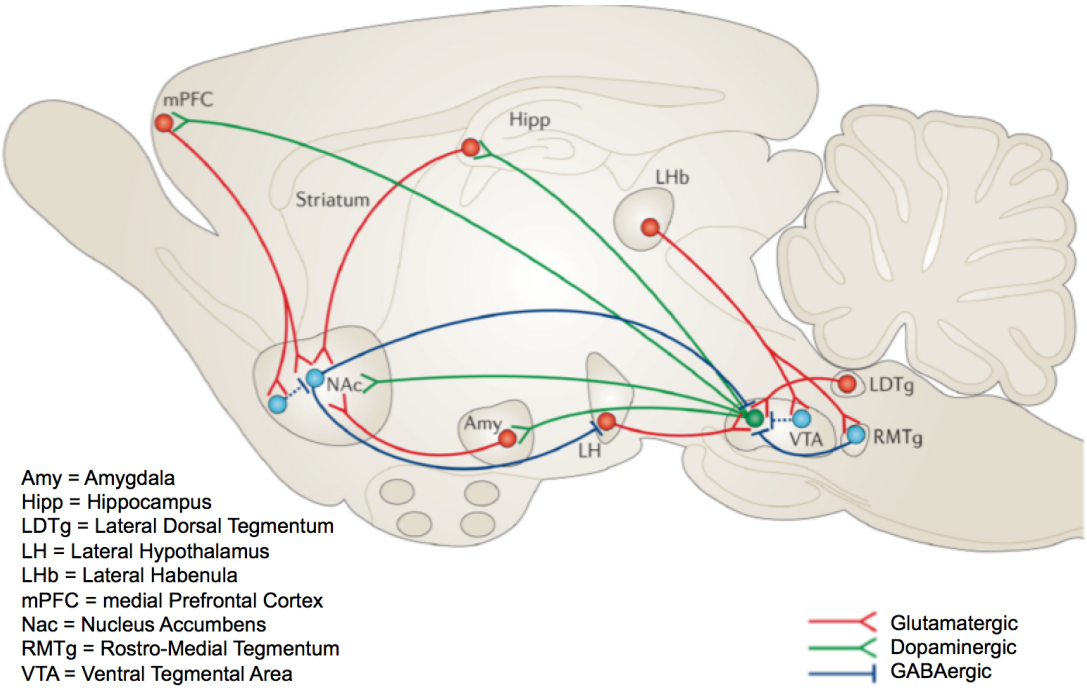



**Figure 4.** A simplified representation of the major connections of the ventral tegmental area (VTA) and nucleus accumbens (NAc). Modified from Russo and Nestler 2013.

The PFCx, for example, is thought to exert cognitive control by computing the relationships between actions and outcomes (Balleine & Dickinson, 1998) and therefore to play a major role in reward expectations, in decision-making, and in delaying gratification. The amygdala and the hippocampus are thought to relay different types of learned associations (emotion-related memories in the case of the amygdala, declarative and spatial memory in the case of the hippocampus).

However, it is important to point out that in addition to the circuitry illustrated in Figure 4, a number of other central and peripheral mechanisms have been shown to be involved, at various levels, in the processing of rewarding stimuli. The response to food stimuli, for example, is modulated by homeostatic signals that can feed onto the meso-telencephalic circuitry, not only indirectly (e.g., via the hypothalamic nuclei, the dorsal raphe nucleus, and the nucleus of the solitary tract) but also directly (e.g., via ghrelin, released from the

gastrointestinal tract, and acting receptors located on dopamine neurons) (see Yeo and Heisler 2012).

Finally, it has been shown that the cerebellum, once thought to play a role only in the control and coordination of movement, it is also involved in a variety of perceptual, cognitive, and emotional functions independent of motor control (Schmahmann 2010; Baumann and Mattingley 2012; Balsters 2013). Indeed, the cerebellum is interconnected with the PFC (BA9 and BA46) via reciprocal pathways, providing an anatomical basis for cerebellar mediation of non-motor "frontal" function including reward processing (Holstege and Georgiadis 2004; Ramnani et al. 2004). In particular, cerebellar activation has been observed in substance abusers while performing reward learning tasks (Anderson et al. 2006; Martin-Solch et al. 2001), experiencing drug craving (Bonson et al. 2002; Olbrich et al. 2006) and, most important for the scope of the present dissertation, when recalling drug-related experiences (Grant et al. 1996).

### 3.2. Dopamine and reward

The literature linking dopamine to reward is enormous, and there is little doubt that meso-accumbens/striatal dopamine neurons play a crucial role in motivated behaviour. Indeed, dopamine concentrations in the terminal regions of this system are increased by all major types of rewarding stimuli, including food (e.g., Di Chiara et al. 1999), sex (e.g., Fiorino and Philips 1997), and addictive drugs (Di Chiara and Imperato 1986). Furthermore, destruction of dopamine neurons produces aphagia and avolition (for a review, see Wise 2008). However, there is still not a general consensus about the exact information encoded by dopamine transmission. This is at least in part the consequence of five major factors.

*3.2.1. Meso-accumbens versus meso-striatal dopamine pathways*

There is evidence that the dopaminergic pathways originating in the VTA and projecting to the NAcc (or ventral striatum) play a more specific role in reward than those originating in the substantia nigra (SN) and projecting to the caudate putamen (or dorsal striatum) (Malenka et al. 2009). However, there is also abundant evidence against this simplification. First of all, the projections of the VTA versus SN are not completely segregated, as many VTA dopamine neurons project also to the caudate putamen and many SN dopamine neurons project also to the NAcc. Furthermore, dopamine neurons projecting to NAcc and dorsal striatum have been reported to respond in a similar manner to unconditioned and conditioned stimuli (UCSs and CSs), as well as cues that control instrumental behaviour (Montague et al. 1996; Morris et al. 2006; Roesch et al. 2007; Schultz 2006; Schultz et al. 1997). Also, rewarding stimuli and cues elicit similar dopamine release in the NAcc and the dorsal striatum (Boileau et al. 2006; Darvas and Palmiter 2010; de la Fuente-Fernández et al., 2002; Kishida et al., 2011; Phillips et al. 2003; Roitman et al. 2004; Volkow et al. 2012; Wanat et al. 2009; Wise 2009; Zaghloul et al. 2009). Finally, rodents learn equally well to emit instrumental behaviour that stimulates dopamine neurons originating in either the VTA or the SN (Witten et al. 2011; Nieh et al. 2013; Rossi et al. 2013).

*3.2.2. Direct versus indirect NAcc/striatal projection pathways*

The neuronal population of the striatal complex includes both interneurons and medium spiny neurons (MSNs), which are GABAergic inhibitory projection neurons. Interneurons represent about 5% of the total number of striatal neurons and express either GABA or acetylcholine. The remaining 95% of

striatal neurons are MSNs that can be further differentiated in those that project to the midbrain directly (and co-express GABA and encephalin) versus those that project indirectly, via globus pallidus and subthalamic nucleus (and co-express GABA, dynorphin and substance P). The MSNs of the direct projection pathway predominantly express dopamine $D_1$ receptors (D1-type MSNs) whereas the neurons of the indirect pathway predominantly express dopamine $D_2$ receptors (D2-type MSNs). A minority or MSNs of the dorsal striatum co-express $D_1$ and $D_2$ receptors, which can heterodymerize (Marcellino et al. 2008).

The direct and the indirect striatal pathways regulate in opposite directions the activity of the output nuclei of the basal ganglia (SN pars reticulata and globus pallidus pars interna), which in turn exert inhibitory control over the motor thalamus. The direct pathway inhibits the output nuclei (thus, disinhibiting the thalamus) whereas the indirect pathway excites the output nuclei (thus, inhibiting the thalamus).

The opposite regulation of the output nuclei of the basal ganglia by the direct versus the indirect pathways translates in the opposite roles of the two pathways in modulating reward and motivation. As shown in Figure 5, for example, mice readily learn to press a lever that triggers optogenetic stimulation of the direct pathway whereas they avoid the lever that stimulates the indirect pathway (Kravitz et al. 2012), suggesting that the direct pathway mediates reward whereas the indirect pathway mediates aversion (even though there is little or no evidence that the projections of the NAcc segregate like those of the dorsal striatum in direct and indirect pathways). Thus, it has been suggested that for produce maximal rewarding effects dopamine increases need to be fast and sufficiently large to stimulate low-affinity $D_1$ receptors in

addition to $D_2$ receptors, leading to the activation of the direct pathway and the inhibition of the indirect pathway.

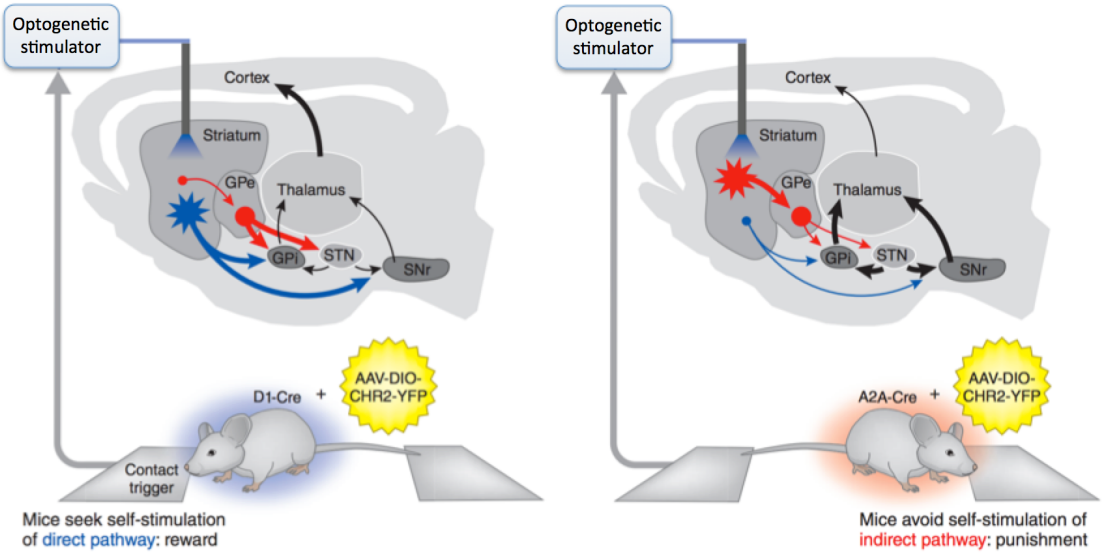



**Figure 5.** Schematic representation of the consequences of the stimulation of the direct versus the indirect projection pathway of the basal ganglia. The light-sensitive cation channel Channelrhodopsin2 (ChR2) was expressed in D1-type MSNs (using D1-Cre transgenic mice, left panel) and D2-type MSNs (using A2A-Cre transgenic mice, right panel) of the dorsomedial striatum. The mice were placed in a chamber with two *manipulanda*. One *manipulandum* triggered a pulse of laser light through the optical fibre. The other *manipulandum* was inactive. Blue: direct pathway originating from D1-type MSNs and impinging directly on the output nuclei of the basal ganglia: Substantia Nigra pars reticulata (SNr) and Globus Pallidus pars interna (GPi). Red: indirect pathway originating from D2-type MSNs and impinging indirectly on the GPi and SNr, via Globus Pallidus pars esterna (GPe) and Subthalamic Nucleus (STN). The thicker the arrow, the greater the relative activity of the projection. Areas outlined in dark grey send inhibitory projections. Areas outlined in white send excitatory projections. The mice self-stimulated the direct pathway (→positive reinforcement) but not the indirect pathway (→punishment). Modified from Paton and Louie 2012.

Stimulation of NAcc $D_1$ receptors, for example, seems to be sufficient for the rewarding effects of cocaine (Caine et al. 2007), whereas $D_2$ receptor stimulation is not (Caine et al. 2002; Durieux et al. 2009; Norman et al. 2011), but maximal reward requires both $D_1$ receptor and $D_2$ receptor activation (Welter et al. 2007).

*3.2.3. Tonic versus phasic dopamine transmission*

Dopaminergic transmission is a very complex phenomenon, as meso-telencephalic dopamine neurons have been shown to fire in at least two distinct modalities (Grace 2016): i) *tonic firing*, consisting of slow, irregular spike activity (1-8 Hz) that produces low dopamine concentrations, and ii) *phasic firing*, consisting of bursts (>15 Hz) of rapid action potential spiking (burst firing) that result in spikes of high dopamine concentration called transients (see Figures 3). The regulation of tonic versus phasic transmission depends on the intrinsic properties of dopamine neurons and on the balance of the various excitatory and inhibitory inputs to the midbrain. Tonic firing of VTA dopamine neurons has been shown to be modulated by afferents from the ventral pallidum and the stria terminals (Georges and Aston-Jones 2001; Mahler et al. 2014), whereas phasic firing involves excitatory and inhibitory afferents from the pedunculopontine tegmentum, the rostromedial tegmental nucleus, the subthalamic nucleus, the laterodorsal tegmentum, the NAcc, the globus pallidus, and the dorsal raphe (Floresco et al. 2003; Lodge and Grace 2006; Paladini and Roeper 2014; Grace 2016). Tonic and phasic dopamine transmissions are thought to play different roles in motivation and reward (Covey et al. 2014; Volkow and Morales 2015; Grace 2016).

The low ambient level of dopamine produced by tonic firing mainly stimulate high-affinity $D_2$ receptors and is thought to support movement, cognition, and motivational drive. In contrast, the spikes of high dopamine concentrations produced by burst firing stimulate low-affinity $D_1$ receptors. Work done by Schultz and coworkers over the past two decades has shown that dopamine transients encode key attributes of natural rewards, such as timing, cost, magnitude, and probability (Schultz 2015). Tonic firing initially occurs in

response to the unexpected presentation of a natural reward, but then its occurrence progressively shifts to the presentation of CSs predicting reward. Dopamine transients also exhibit the requisite temporal precision and amplitude to promote plasticity of cortico-striatal synapses that is associated with reward learning (Reynolds et al. 2001; Arbuthnott and Wickens 2007). The exact information encoded by dopamine transient is still debated, but two main conceptual models have emerged. According to one model phasic dopamine transmission encodes a "reward prediction error" (Schultz 2015). That is, unexpected or greater than expected rewards increase burst firing, thus reinforcing behaviour, whereas expected rewards do not produce burst firing and absent or worse than expected rewards decrease burst firing, thus suppressing behaviour. According to a second model (discussed in the next section), phasic dopamine transmission attributes 'incentive salience' to reward predicting cues and generate subjective craving and appetitive behaviour.

As a corollary to the points discussed above, it is important to point out that teasing out the exact role of dopamine in reward based on findings from the literature is complicated not only by differences in the experimental paradigm(s) employed, but also by differences in the temporal resolution of the various methodologies used to monitor and quantify dopamine transmission, which range from the sub-second scale (e.g., fast scan cyclic voltammetry, electrophysiology) to the >1 minute scale (e.g., microdialysis, positron emission tomography imaging of dopamine receptors). This makes it difficult to compare findings from studies using different methodologies, as the changes in dopaminergic activity measured using fast scan cyclic voltammetry and electrophysiology probably reflect the phasic firing of dopamine neurons,

whereas the changes measured using microdialysis and positron emission tomography (PET) imaging reflect their tonic firing.

*3.2.4. Competing models of reward and motivation*

The fourth, and probably most important, issue in complicating the interpretation of the role of the dopaminergic system in reward is represented by competing models of reward based on different definitions of the term itself and on the parsing of different subcomponents. For the purposes of this dissertation, two models will be examined. The first model (Figure 6) was initially proposed by Dalbir Bindra (1959, 1974) and then formalized by Toates (1986). According to this model, the reward value of an unconditioned stimulus (UCS) is processed by specialized brain reward circuitry that elicits a hedonic response and assigns incentive salience to it. Conditioned stimuli (CSs) can acquire, via associative learning, the ability to access the brain reward circuitry and produce effects similar to those produced by the UCS. More recently, Kent Berridge and colleagues at the University of Michigan proposed a different model of reward and motivation (Robinson and Berridge 1993; Berridge et al. 2009; Berridge 2004, 2007, 2012) that builds on the Bindra-Toates model but distinguishes two major sub-components (Figure 7): pleasure (the so-called 'liking') and incentive salience (the so-called 'wanting'). Thus, in the Michigan model, the neurobiological substrates of wanting (which produce subjective desire, attracts the animal towards stimuli, and elicit consumption) are segregated from those responsible for liking (which produce the subjective experience of pleasure as well as unconscious positive affective states).

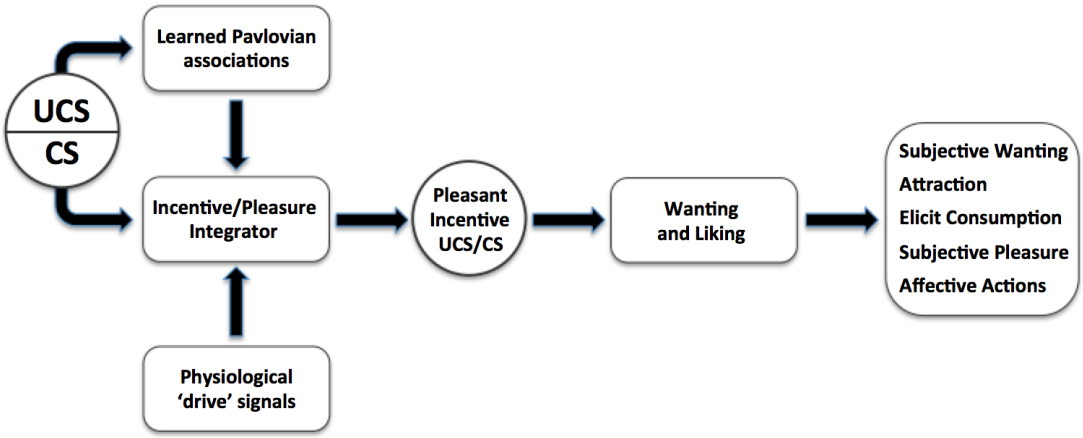



**Figure 6**. Bindra-Toates' model of reward and motivation, according to Robinson and Berridge (1993).

Although UCSs and CSs usually trigger parallel changes in wanting and liking, so as to appear inextricably bundled, under certain conditions the two processes can be dissociated.

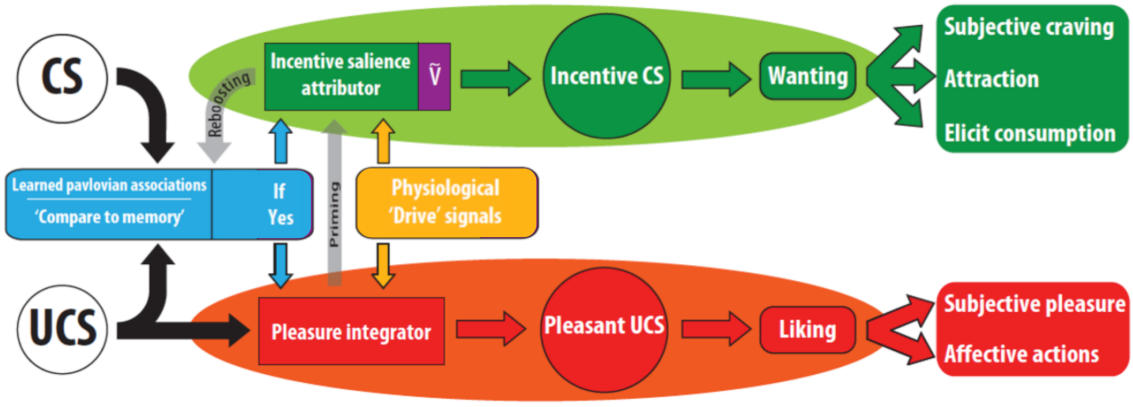



**Figure 7.** The Michigan model of reward and motivation (Robinson & Berridge 1993). Modified from Berridge (2012).

The two models have powerful implications for the attribution of a specific role to dopamine in reward. Many researchers follow, sometimes unwittingly, one version or the other of the Bindra-Toates model and assume that subjective or unconscious pleasure is associated to all rewarding UCSs or CSs. Hence, dopaminergic transmission is thought to encode phenomena such

as euphoria and 'yumminess', a notion that has been very popular in the 1980s and 1990s, especially in the field of drug abuse research (Wise 1980, 2008). However, in the past two decades, a wealth of animal and human studies have shown that the hedonia/anhedonia dopamine hypothesis is incompatible with experimental findings (Berridge 2012; Berridge and Kringelbach 2013). In particular, it has been shown that lesions of the dopamine neurons or pharmacological blockade of dopamine receptors have little or no effects on the 'liking' component of reward.

By contrast, according to the Michigan model, dopamine encodes the 'wanting' component of reward but not the 'liking' one. That is, in this model the dopaminergic meso-accumbens/striatal system (and related circuitry) serves as the 'incentive salience attributor' whereas distinct brain mechanisms are implicated in the hedonic experience (Berridge and Kringelbach 2013).

*3.2.5. The role of dopamine in reward versus aversion*

Finally, it is important to point out that it has been known since the early 1990s that restraint stress can increase dopamine levels in the NAcc of the rat (Imperato et al. 1991; Puglisi-Allegra et al. 1991), indicating that the mesolimbic dopamine system can be activated not only by rewarding stimuli but also by aversive stimuli. Interestingly, Puglisi-Allegra and colleagues found that dopamine levels increased at the beginning of restraint and then again when the rats were freed. However, the early dopamine response rapidly adapted after repeated exposure to restraint stress, whereas the increase in dopamine produced by the release from restraint remained unchanged (Imperato et al. 1992). Puglisi-Allegra and colleagues concluded "that the activation of the mesolimbic dopaminergic system induced by aversive stimuli adapts to

repeated experiences differently from that produced by pleasurable events, suggesting that aversive and rewarding experiences involve different neural systems." Indeed, it has been shown that VTA dopamine neurons are anatomically and functionally heterogeneous projecting to different sub-regions of the NAcc. Lammel and colleagues (2014) have recently reviewed the literature concerning the different sub-circuits of the mesolimbic dopamine system and their differential role in mediating reward versus aversion. However, much remains to be done to clarify this topic in order to "explain a number of previously confusing observations that suggested a role for DA in processing both rewarding as well as aversive events" (Lammel et al. 2014, page 351).

### 3.3. Neurobiological substrates of heroin versus cocaine reinforcing effects

#### *3.3.1. Shared mechanisms of action of addictive drugs*

*3.3.1.1. Animal studies.* Despite the very different primary mechanisms of action of heroin versus cocaine, there is evidence of partial overlap in the more distal neurobiological effects of the two drugs, particularly at the level of the meso-accumbens/striatal circuitry. Indeed, as discussed in the previous section, the prevailing trend for the past three decades has been to focus on shared mechanisms of drug reward, and in particular on the meso-accumbens dopaminergic system. It is widely thought that not only psychostimulants but also MOR agonists, as well as nicotine, alcohol, and cannabinoids, impinge directly and/or indirectly onto meso-accumbens dopamine neurons, even though "these actions may differ across heterogenous subsets of midbrain dopamine neurons" (for a recent review, see Covey et al. 2014). The final outcome in all cases is represented by increased dopamine concentrations and

increased dopamine transmission as illustrated in Figure 8. As discussed in previous sections, cues that via associative learning acquire the ability to predict drug rewards are also thought to act mainly via the phasic activation of dopaminergic transmission.

In the case of cocaine and other psychostimulant drugs (such as amphetamine and methamphetamine) the mechanisms responsible for their ability to facilitate dopaminergic transmission is easily identified in their actions on the DAT (see Section 2) and the consequent increase in dopamine levels in the terminal regions of the mesolimbic system. In contrast, the mechanisms implicated in the effects of heroin and other drugs are more complex.

Heroin and other opioid drugs are thought to disinhibit DA neurons by acting on MOR located on GABAergic neurons that impinge on mesolimbic dopaminergic neurons (see Figure 8). The most important evidence in support of a major role of the mesolimbic dopaminergic system in mediating the effects of heroin can be summarized as follows: i) the VTA contains dense concentrations of both MOR and KOR (Greenwell et al. 2002; Sesack and Pickel 1992; Bausch et al. 1995; Garzon and Pickel 2001); ii) systemic and intra-VTA administration of MOR agonists increase dopamine release in the ventral striatum (Spanagel et al. 1992; Devine et al. 1993; Yoshida et al. 1993; Di Chiara and Imperato 1986; Chefer et al. 2003); iii) systemic or intra-VTA administration of morphine in anaesthetized animals increase the firing rate of putative dopamine neurons, (Gysling and Wang 1983; Melis et al. 2000; Jalabert et al. 2011; Kiyatkin and Rebec 2001; Matthews and German 1984); iv) the selective MOR agonist DAMGO activates putative VTA dopamine neurons in ex vivo preparations (Johnson and North 1992; Kiyatkin and Rebec 1997, 2001).

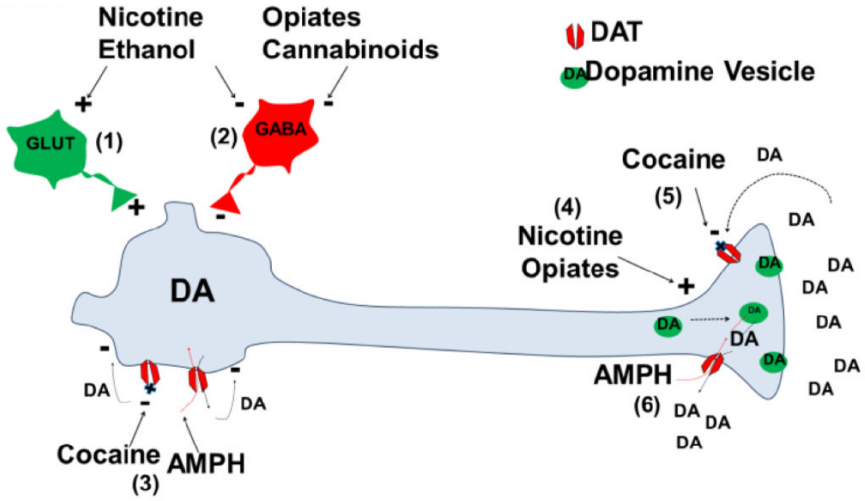



**Figure 8.** All addictive drugs are though to impinge on midbrain dopamine neurons (DA), either directly or indirectly, via glutamatergic neurons (GLUT, 1) or GABAergic neurons (GABA, 2). Facilitation and inhibition of neuronal activity are indicated by the positive (+) and negative (-) signs, respectively. Opiates are thought to disinhibit dopamine neuron by reducing the inhibitory action of GABAergic neurons (2). Furthermore, opiates up-regulate DA release by increasing the amplitude of phasic relative to tonic dopaminergic activity (4). In contrast, both amphetamine (AMPH) and cocaine bind the DA transporter (DAT) thereby increasing the concentrations of DA, which in turn suppresses DA neurons firing by acting on D2 autoreceptors located on the cell body (3) or on the terminals (5). Thus, opiates increase DA levels by increasing DA neuron activity, whereas psychostimulants increase DA levels, despite reducing DA neuron activity. Modified from Covey et al. (2014).

Yet, it is important to point out that there are findings that are not consistent with the notion that heroin per se increases dopaminergic transmission. Kiyatkin and colleagues, for example, found that while passive injection of heroin increases the firing rate of putative VTA dopamine neurons in anaesthetized animals (Kiyatkin and Rebec 2001), passive or self-administered injection of heroin in awake, drug-naïve rats, decrease firing. In particular, Kiyatkin and Rebec showed that the firing rate decreased immediately following each self-administration of heroin, slowly recovering and peaking just before the next self-administration (Kiyatkin 1994; Kiyatkin and Rebec 2001). However, as pointed out by Fields and Margolis (2015), the criteria used to identify VTA neurons as dopaminergic (e.g., dopamine $D_2$ receptor inhibition, action potential duration, or firing pattern) in the in vivo

electrophysiological studies conducted so far are not reliable (Lammel et al. 2008; Margolis et al. 2006, 2012; Cohen et al. 2012). Only direct identification of the neurotransmitter content of recorded neurons (for example, see Cohen et al. 2012) in awake animals would allow to determine the effect of heroin and other opioid agonists in rodents.

*3.3.1.2. Human studies.* The effects of addictive drugs on dopamine transmission has also been investigated in humans studies using PET imaging of dopamine $D_2$ receptors with labeled radiotracers such as [$^{11}$C]raclopride. Increased dopamine transmission has been consistently found after administration of cocaine (e.g., Schlaepfer et al. 1997; Cox et al. 2011), amphetamine (e.g., Laruelle 1995; Breier et al. 1997; Leyton et al. 2002, 2004), or methylphenidate (e.g., Volkow et al. 1999, 2001, 2008, 2014).

In contrast, at present, there is no evidence of enhanced dopaminergic transmission after administration of heroin or other opioid agonists in humans (Nutt et al. 2015). In particular, the only two studies (to the best of my knowledge) in which dynamic PET imaging was used in conjunction with heroin administration no change of dopamine receptor binding was observed in any sub-region of the striatal complex at any point in time during the scan (Daglish et al. 2008; Watson et al. 2013). Furthermore, no change in dopamine receptor binding was observed after the presentation of heroin-paired cues that were sufficient to elicit a positive affective state (Watson et al. 2013). It is possible that these negative findings might be due to methodological limitations, but it is nevertheless striking that the same technique (i.e., dynamic PET imaging with [$^{11}$C]raclopride) has allowed researchers to identify changes in dopamine transmission following administration of psychostimulant drugs

(or exposure to psychostimulant-associated cues) but not after administration of heroin (or exposure to heroin-associated cues).

### *3.3.2. Shared substrates of drug reward*

*3.3.2.1. Animal studies.* Evidence from rodent studies indicates that dopaminergic transmission is required for the reinforcing effect of cocaine and other psychostimulant drugs (for reviews, see Wise 2008; Badiani et al. 2011). In contrast, the exact role of the dopaminergic system in mediating the direct rewarding effects of non-psychostimulant drugs is less clear than what is usually thought, as repeatedly pointed out by Badiani and colleagues (Badiani et al. 2011; Badiani 2013, 2014; Badiani et al. 2017).

In particular, there is experimental evidence from a few comparative studies that disruption of dopamine transmission affects the reinforcing effects of cocaine but not those of heroin. Studies from the early 1980s have shown in fact that administration of the dopamine receptor antagonist alfa-flupentixol (Ettemberg et al. 1982) or 6-hydroxy-dopamine lesions of the mesolimbic dopamine system (Pettit et al. 1984) severely impair cocaine self-administration but not heroin self-administration. Similar findings were reported by others (Dworkin et al. 1988; Gerrits et al. 1996). More recently, Pisanu and colleagues (2015) found that silencing the RNA encoding for dopamine $D_1$ receptors in the shell of the nucleus accumbens completely blocks the acquisition of intravenous cocaine self-administration but has no effect on the acquisition of heroin self-administration.

*3.3.2.2. Human studies.* Also in humans, there is some evidence linking the enhancement of dopaminergic transmission produced by psychostimulants to subjective measures of reward (e.g., Schlaepfer et al. 1997; Cox et al. 2011), amphetamine (e.g., Laruelle 1995; Breier et al. 1997; Leyton et al. 2002, 2004). In contrast, it has been shown that heroin can produce its characteristic euphoriant effect in humans without producing any change in dopamine transmission in the striatum, as indicated by dynamic PET imaging with the labeled radiotracer [$^{11}$C]raclopride (Daglish et al. 2008; Watson et al. 2013). Interestingly, also the expectation of heroin reward despite producing a positive affective state failed to alter dopamine receptor binding (Watson et al. 2013).

*3.3.3. Summary*

Evidence from both animal and human studies suggest that the neurobiological substrates of the direct rewarding effects of cocaine are at least partly different from those of heroin. More specifically, it appears that while dopaminergic transmission plays a major role in psychostimulant reward, this is not the case for opiate reward. The importance of these apparent discrepancies is obvious, given that (as discussed in the first section of this chapter) most theoretical models of reward, including drug reward, posit the existence of shared substrates that are both necessary and sufficient to explain the direct reinforcing effects of rewarding stimuli.

It is possible, that, as already noted above, methodological limitations and differences in testing procedures might account for the discrepancies observed between opiate and psychostimulant drugs. In this respect, it is important to point out that very few comparative studies including more than one class of addictive drugs have been conducted in the past decades. Most of

the experimental evidence comes in fact from studies with cocaine or amphetamine. However, it striking that the few comparative studies with cocaine and heroin consistently show major differences in the direct rewarding effects of these two drugs, as in the case of the rodent studies that investigated the role of dopaminergic transmission in cocaine vs. heroin self-administration (Ettenberg et al. 1982; Pettit et al. 1984; Dworkin et al. 1988; Gerrits et al. 1996). Additional evidence of dissociation between cocaine and heroin reward has been provided by the studies by Badiani and colleagues that will be reviewed in Section 4 of this chapter.

## 4. Substance-specific environmental influences on the rewarding effects of cocaine and heroin

As discussed in the previous sections, heroin and cocaine act on specific binding sites in the reward regions of the brain. However, it has long been noted that the rewarding effects of addictive drugs are not a simple consequence of their primary neuropharmacological actions. A number of pharmacological and non-pharmacological factors contribute to shape the individual response to drugs. Previous drug exposure, for example, can induce neuroplastic changes in brain reward areas and related areas that alter the response to subsequent exposure to the same drug as well as to other drugs (for reviews, see Stewart and Badiani 1993; Badiani and Robinson 2004; Robinson and Kolb 2004). The nature of these neuroplastic adaptations and their localization varies as a function of the type of drug and of the schedule of drug administration. Also, exposure to adverse life events can produce neuroplastic

changes that affect drug responsiveness (for a review, see Caprioli et al. 2007b). More in general, the environment can exert its influence on individual organisms throughout their life span thereby changing continually their phenotype. It is on these unique phenotypes that drugs act. That is, the individual response to a drug should be seen as the result of an interaction between the drug and a unique *set* of phenotypic characteristics.

The notion that individual variability in drug responsiveness is not a simple function of genotype but is also due to environmental influences is not new and it is safe to say that no one in the field of neuropsychopharmacology would seriously object to it. The same can also be said for the notion of individual differences in the vulnerability to drug addiction. A series of seminal studies by Tsuang and colleagues (1998, 1999, 2001) and then by Kendler and colleagues (2003, 2007) have clearly shown the extent to which the propensity to initial drug use and the transition from use to abuse is modulated by environmental influences. In contrast, less attention has been paid to two important findings of the studies mentioned above. The first one is that the role of environmental factors weighs more heavily on the susceptibility to heroin abuse than on the susceptibility to cocaine abuse. The second finding offers a clear answer to "a central question in the etiology of drug abuse", that is, "the extent to which the risk factors for the use or misuse of a particular class of psychoactive substances are specific to that class or are nonspecific in that they predispose the individual to the use or misuse of a wide range of such compounds" (Kendler et al. 2003, page 687). Kendler and colleagues found that "environmental experiences unique to the person largely determine whether predisposed individuals will use or misuse one class of psychoactive substances rather than another" (Kendler et al. 2003, page 687). In particular, it appears

that there are environmental factors that are capable to facilitate preferentially heroin but not cocaine abuse and vice versa. However, the exact nature of these substance-specific environmental influences is still not known, and even less is known about the relative neurobiological substrates.

One way to begin addressing this issue would be to identify a relatively simple environmental manipulation, amenable to experimental investigation and capable to affect drug responsiveness in a substance-specific manner. Most research done so far has focused almost exclusively on the ability of acute and chronic stressors and of associative learning processes to shape the individual *set*, thereby affecting drug responsiveness. However, there is no evidence suggesting that CSs or stressors exert their modulatory influence in a substance-specific manner, that is, by facilitating the reinforcing effects of one class of drugs but not of others. In contrast, a series of studies published in the last ten years have shown that the surroundings of drug taking (the *setting*) can have a powerful and, most important, substance-specific influence on the rewarding effects of heroin versus cocaine in both rodents and humans (Badiani 2013). In the next sections these studies will be reviewed in detail, as they provide the rationale for the experiments described in the present dissertation.

### 4.1. Drug, set and setting

During the Vietnam War more that 40% of American soldiers made use of opioid drugs and about 20% became addicted, mostly to heroin (Robins 1975). Yet, once back in the United States only a minority of these veterans continued to abuse heroin. As later pointed out by Robins (1993) "the surprisingly low levels of re-addiction and the rarity of addiction to narcotics alone as compared with poly-substance dependence are findings still not entirely incorporated into

public and scientific views of heroin addiction." One of the few scholars to propose an explanatory framework for this phenomenon was Norman Zinberg. In an influential book titled *Drug, Set, and Setting* (1984), Zinberg argued that drug-taking behaviour is the result of an interaction among: 1) the pharmacological properties of the *drug*; (2) the attitudes and personality of the user (the *set*); and (3) the social and physical environment in which use occurs (the *setting*). Clearly, what had changed in the life of the Vietnam veterans was not the drug or their personality but the setting of drug use.

Zinberg was concerned mainly with the topic of controlled drug use and with the legal and clinical implications of his novel approach to the problem. However, the notion of an interaction among drug, set, and setting has clearly powerful implications also for the study of the neurobiological bases of drug reward and drug abuse. Nevertheless, of the >1,140 citations received by Zinberg's book (source: Google Scholar, 31 July 2017), only a small minority comes from neuroscience publications and often in a very cursory manner. It is fair to say that the field of neuropsychopharmacology has still not fully incorporated the innovative perspective proposed by Zinberg.

Of course, one of the major obstacles to the investigation of drug-set-setting interactions under controlled conditions comes from the difficult of modeling the *setting* in the laboratory. However, a few attempts at reproducing discrete features of the context of drug taking in animal models have been made by laboratories in Italy and France and USA. Some of these attempts have focused on providing rats with alternative rewards such as food (Lenoir and Ahmed 2008; Caprioli et al. 2015). Others approaches have focused on manipulating the 'psychological' features of the setting of drug use (see Badiani

2013). In the next two sections, I will discuss the findings obtained with this last type of animal model and on the translational studies stemming from it.

### 4.2. Experimental investigation of the setting of drug taking

Humans can take drugs in a variety of settings, such as the user's own home, friends' home, pubs, clubs, etc. Zinberg has argued quite convincingly that the behavioural and subjective effects of a drug can change as a function of the social and physical characteristics of a specific setting. However, these settings differ not only in social and physical terms but also from the point of view of the 'psychological' meaning of the setting for the individual. For example, a given apartment might represent the home environment for one person but a non-home environment for another, even though the setting is identical from a social and physical point of view. To investigate this feature of the setting of drug use in an animal model, Badiani and colleagues adapted an earlier model developed in the early 1990s in the laboratory of Terry Robinson at the University of Michigan.

#### *4.2.1. An animal model of setting*

An initial series of studies concerning the role setting in modulating drug responsiveness in the rat were conducted using psychomotor activity as a proxy for the effects of drugs on the activity of the mesolimbic dopaminergic system, and psychomotor sensitization as an index of neuroplasticity in the same system (for reviews, see Robinson et al. 1998; Badiani and Robinson 2004; Caprioli et al. 2007b). These studies demonstrated that the magnitude of the acute and sensitized psychomotor responses to amphetamine, cocaine, morphine, and heroin were smaller when the drugs were administered to rats

that lived in the test chambers (Home group or Resident group) relative to rats that were transferred to physically identical chambers only for the test sessions (Novel group or Non-Resident group) (Badiani et al. 1995a, 1995b, 1997, 2000a; Crombag et al. 1996; Browman et al. 1998a, 1998b; Fraioli et al. 1999; Ostrander et al. 2003; Paolone et al. 2003, 2007). It is important to note that the setting of drug administration did not alter all drug effects in the same way. Paolone and colleagues (2003), for example, demonstrated that morphine-induced psychomotor sensitization was facilitated in Non-Resident rats relative to Resident rats whereas the prophagic effect was only partly affected and the development of tolerance to the analgesic effect was not affected at all. Finally, two studies demonstrated that the ability of environmental context to facilitate psychomotor sensitization to amphetamine could be dissociated from its effect on acute drug responsiveness and on conditioned responding (Crombag et al. 2000, 2001).

Interestingly, the modulatory effect of setting on drug-induced psychomotor activity did not seem to depend (at least in the case of amphetamine) on differences in pharmacokinetics (Badiani et al. 1997), on the involvement of the hypothalamo-pituitary-adrenal (HPA) axis (Badiani et al. 1995c), or on the facilitation of dopamine release in the NAcc and other sub-regions of the striatal complex (Badiani et al. 1998, 2000b). In contrast, an interaction between drug and setting was observed downstream from dopaminergic transmission in the medium spiny neurons of the striatum (Badiani et al. 1998, 1999; Ostrander et al. 2003; Paolone et al. 2007) and in the amygdala (Day et al. 2001), as indicated by the expression of the transcription factor Fos or of Fos-mRNA.

The modulatory influence of setting did not simply change the magnitude of drug-induced Fos expression; most importantly, it changed the pattern of neuronal activation. In Resident rats cocaine or amphetamine increased Fos-mRNA expression only in D1-like MSNs, whereas in Non-Resident rats these drugs increased Fos-mRNA expression also in D2-like MSNs (Badiani et al, 1999, Uslaner et al. 2001a, 2001b; Ferguson et al. 2003; Hope et al. 2006). Also morphine administration resulted in differential activation of D1- versus D2-like MSNs as a function of setting (Ferguson et al. 2004) but with a pattern quite different from that observed for cocaine and amphetamine, as morphine *decreased* Fos-mRNA expression in D2-like MSNs. Another brain area where the setting altered the activating effects of opioid drugs in a different manner than for psychostimulants was the somatosensory cortex. In this brain region, Ostrander and colleagues (2003) found no effect of setting on the response to repeated administrations of amphetamine. In contrast, Paolone and colleagues (2007) found a significant interaction when the same experiment was conducted with heroin.

In summary, using the animal model of setting described above it was found that psychomotor sensitization to both psychostimulant and opioid drugs was facilitated when drugs were administered outside the home environment relative to the home environment. However, the setting seemed to affect in a different manner the neurobiological response to psychostimulant versus opioid drugs. These findings were obtained with non-contingent drug administrations (that is, the drugs were administered by the experimenter). As discussed in the following section, the same environmental manipulation was later adapted to study the influence of setting on the rewarding effects of self-administered cocaine, amphetamine, and heroin.

*4.2.2. Substance-specific influence of setting on drug use: Animal studies*

Drugs can act as reinforcing stimuli not only in humans but also in animals, including the laboratory rat. Rats with intravenous catheters spontaneously work to self-administer the same addictive drugs taken intravenously by humans: cocaine, amphetamine, methamphetamine, heroin, etc. (for reviews, see Mello and Negus 1996; Haney and Spealman 2008). A typical apparatus for drug self-administration experiments consists of a chamber equipped with an 'active' lever, the pressing of which activates an electronic pump that delivers, via a syringe connected with the catheter, a bolus of drug into the jugular vein of the rat. An 'inactive' lever (the pressing of which does not trigger drug delivery) is often used as a control *manipulandum*. Depending on the specific aim of the study, experimental protocols can vary greatly in terms of: i) *manipulanda* (levers, holes for nose-poking, etc.); ii) availability of contextual and discrete cues paired with drug delivery; iii) schedule of drug reinforcement; iv) duration and frequency of drug availability; v) speed of drug delivery; and a variety of other parameters. By using appropriate combinations of drug self-administration parameters, researchers have been able to investigate the role of CSs in controlling drug taking, the motivation to take the drug, the resistance to extinction of drug-taking behaviour, and other important information.

However, drug self-administration in the rat represents, *per se*, simply an animal model of drug use and does not necessarily provide information about the mechanisms responsible for the development of drug addiction. Indeed, several animal models have been developed to reproduce, with different degrees of face validity, some of the most relevant features of drug addiction.

Hence, to investigate the interaction between drug and setting in drug reward and addiction, Badiani and colleagues combined the animal model of setting, already described in previous section, with animal models of drug taking and of relapse first proposed by Stewart and colleagues (Shaham et al. 2003), as illustrated in the upper panel of Figure 9. In addition, Badiani and colleagues developed an animal model of drug choice in which rats were given the opportunity to choose between different types of drugs within the same experimental session. To this end, rats received double-lumen catheters and the self-administration apparatus was programmed so that by pressing on one lever or the other of the self-administration apparatus a rat was able to choose whether to self-administer one drug (e.g., cocaine) or another (e.g., heroin). A synopsis of the main results of the studies by Badiani and colleagues is illustrated in Table 1.

Table 1.

| | Heroin | Cocaine |
|---|---|---|
| **Intake**<br>Caprioli et al. 2007a, 2008, 2009<br>Celentano et al. 2009 | Rats take more heroin at home than outside the home | Rats take more cocaine outside the home than at home |
| **'Motivation' (PR)**<br>Caprioli et al. 2007a, 2008<br>Celentano et al. 2009 | Rats are willing to work harder for heroin at home than outside the home | Rats are willing to work harder for cocaine outside the home than at home |
| **Choice: Heroin vs. Cocaine**<br>Caprioli et al. 2009<br>De Luca et al., in preparation | Rats tend to choose heroin at home | Rats tend to choose cocaine outside the home |
| **'Pleasure' (50 kHz USVs)**<br>Avvisati et al. 2016 | Heroin 'pleasure' is greater than cocaine pleasure at home | Cocaine 'pleasure' is greater than heroin pleasure outside the home |
| **'Craving' after abstinence (Relapse)**<br>Montanari et al. 2015 | Rats relapse into heroin seeking at home but not outside the home | Rats relapse into cocaine seeking outside the home but not at home |

Overall, it can be seen that the setting consistently modulated in opposite direction the response to heroin versus cocaine. Resident rats took more heroin than Non-Resident rats (Figure 9, bottom panel) and worked harder (as indicated by progressive ratio schedule of reinforcement) for it. The opposite was observed for cocaine and amphetamine (Caprioli et al. 2007a, 2008; Celentano et al. 2009).

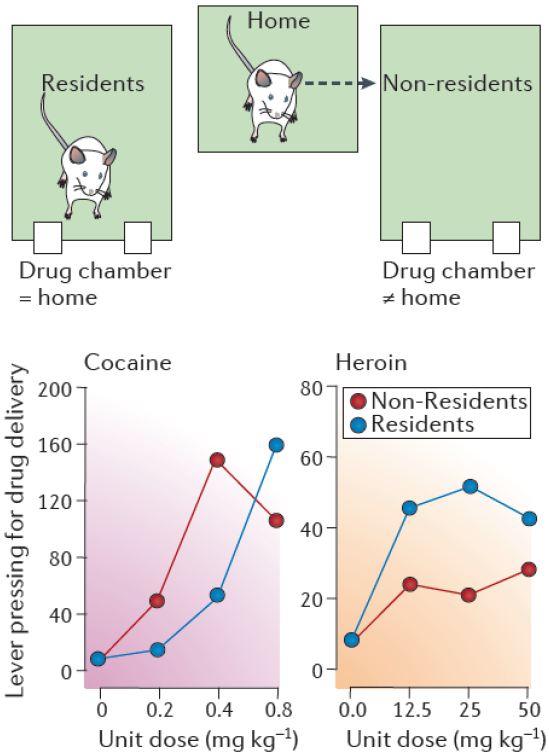



**Figure 9.** Drug taking as a function of setting in the rat: Some rats were transferred to standard two-lever self-administration chambers (one lever paired with drug infusions, the other lever inactive) immediately before the sessions (Non-Resident rats), while other rats were kept in these chambers at all times (Resident rats). Heroin was more rewarding in the Resident rats than in the Non-residents rats (indicated by an upward and left shift in the dose-response curve). In contrast, cocaine was more rewarding in the Non-resident rats than in the Residents rats (indicated by a left shift in the dose-response curve). Modified from Badiani et al. 2011.

Furthermore, rats with double-lumen catheters that had been trained to self-administer both cocaine and heroin and were then given the opportunity to choose between the two drugs, made their choice as a function of setting (Figure 10). The majority of Resident rats preferred heroin to cocaine whereas

Non Resident rats tended to prefer cocaine to heroin (Caprioli et al. 2009). Resident rats also emitted more 50 kHz ultrasonic vocalizations (USVs) in response to heroin than to cocaine, and the opposite was observed for Non-Resident rats (Avvisati et al. 2016). It has been proposed that that 50 kHz USVs reflect the positive affective valence of drug experience in the rat, as they are emitted in response to presumably rewarding stimuli, including food, sex, tickling, playing, and addictive drugs (Knutson et al. 1998; Panksepp and Burgdorf 2000; White et al. 1990; McGinnis and Vakulenko 2003; Barker et al. 2010). Thus, it seems that the setting modulates in opposite direction not only the intake of and the motivation for heroin versus cocaine but also the affective response to the two drugs.

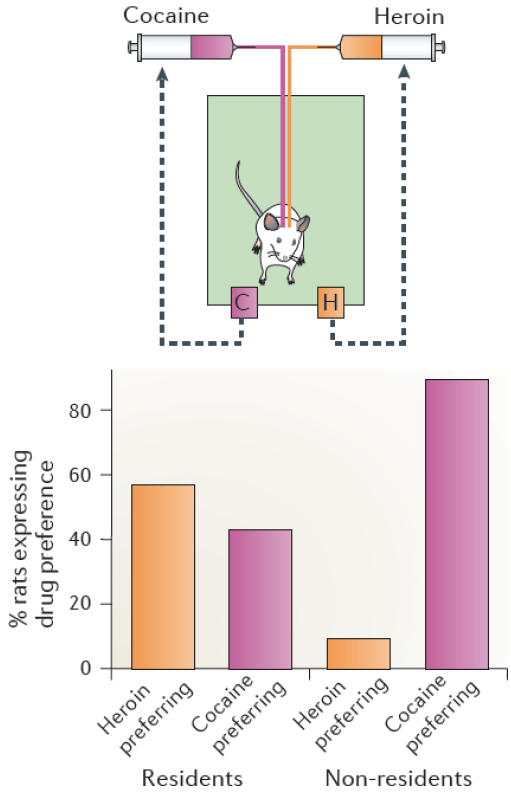



**Figure 10.** Drug preference as a function of setting in the rat: Resident and Non-Resident rats with double-lumen catheters were first trained to self-administer heroin and cocaine on alternate days and were then given the opportunity to choose between cocaine and heroin within the same session. Most Resident rats preferred heroin over cocaine whereas most Non-Resident rats preferred cocaine over heroin. Modified from Badiani et al. 2011.

In addition to modulating drug use, the setting exerted a powerful influence on the vulnerability to relapse into drug seeking after abstinence (Montanari et al. 2015). Rats with double-lumen catheters were trained to self-administer both cocaine and heroin on alternate days and then underwent a 10-day extinction procedure during which upon completion of each instrumental task they received vehicle (paired with the appropriate drug cue) instead of the drug solution. After extinction the rats were tested in a reinstatement procedure at the beginning of which they received a single 'priming' intravenous infusion of different doses of either heroin or cocaine. Remarkably, only Resident rats relapsed into heroin seeking, as indicated by non-reinforced responding on the 'heroin lever', whereas only Non-Resident rats relapsed into cocaine seeking, as indicated by non-reinforced responding on the 'cocaine lever' (Figure 11). These findings indicate that the internal state produced by heroin and cocaine may precipitate drug craving in some settings but not in others, suggesting that a 'lapse' into drug use may lead to actual relapse into drug addiction in some settings but not in others.

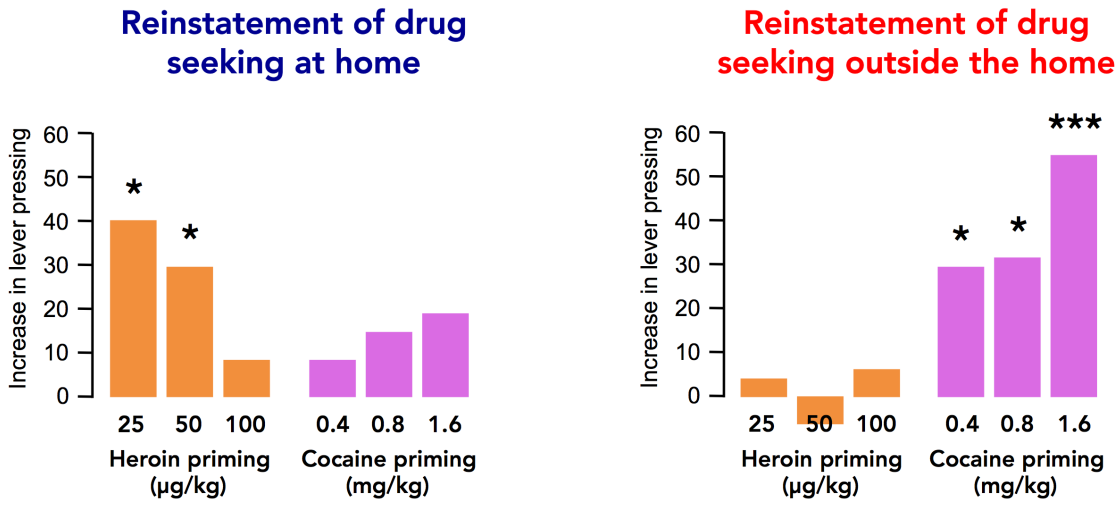



**Figure 11.** Reinstatement of heroin versus cocaine seeking as a function of the setting of drug priming. Data from Montanari et al. (2015).

In summary, the same physical setting can acquire, by a very simple manipulation of its 'psychological' meaning (i.e., home versus outside the home environment), the ability to influence in opposite manner the rewarding properties of cocaine and heroin, including their ability to trigger relapse into drug seeking in rat model of relapse. The neurobiological basis of this phenomenon are not known but experiments conducted with repeated non-contingent drug administrations suggest that the setting modulates in a very different manner the effects of opioids and psychostimulants on the activity of the striatal complex and the somatosensory cortex. These striking findings led to conduct the translational studies described in the next section.

*4.2.3. Substance-specific influence of setting on drug use: Human studies*

Building on the findings from the animal studies described above, Badiani and colleagues conducted a series of studies in human addicts to investigate the setting of drug taking for heroin versus cocaine, using retrospective reports (Caprioli et al. 2009; Badiani and Spagnolo 2013). Heroin and cocaine co-abusers, recruited among the outpatients of a public drug addiction clinic in Rome (Italy), were interviewed about the circumstances of drug taking in the previous three months. The criteria for recruitment included: i) DSM-IV-R criteria for cocaine and/or heroin dependence; ii) no other major psychiatric disorder; iii) a fixed address. The participants were also asked to specify whether the setting of drug use represented a real preference or was the result of constraints. They all confirmed that their choice of setting was a real preference.

As illustrated in Figure 12, the addicts reported distinct setting preferences for the two drugs: heroin was used exclusively or preferentially at

home whereas cocaine was used exclusively or preferentially outside the home. These data concern only the separate use of heroin and cocaine in distinct occasions. Of the few participants who reported injecting heroin and cocaine in combination (speedball), 70% preferred exclusively the home setting, whereas 23% always preferred a non-home setting.

The data were also analyzed in subgroups of addicts using different routes of administration. Regardless of whether the addicts injected both drugs intravenously or took both drugs via insufflation, setting preferences were the same. This suggests that setting preferences were not the result of practical constraints associated with the rituals of drug injection or of snorting. Most important, these setting preferences did not appear to depend on the social features of the setting because they were evident also in addicts who took both drugs in the company of others.

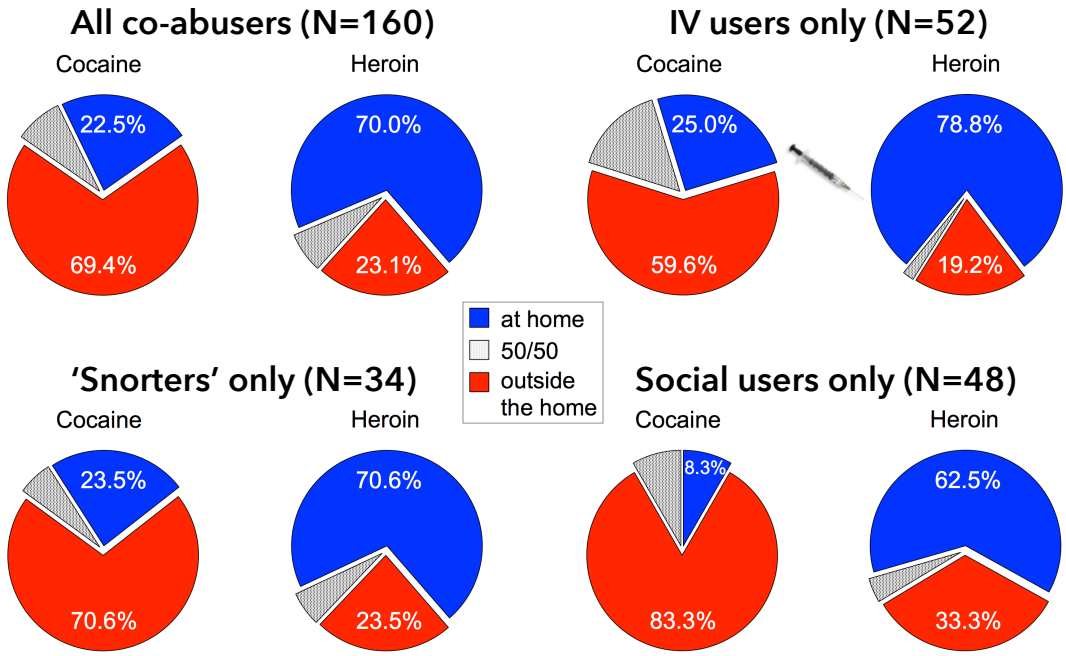



**Figure 12.** Setting Preferences for cocaine vs. heroin use in co-abusers (N=160). Data from Caprioli et al. (2009) and Badiani and Spagnolo (2013).

*4.2.4. Summary*

Both animal and human studies indicate that the setting of drug use exerts a powerful and substance specific influence on drug reward. Specifically, heroin was more rewarding than cocaine when rats self-administered these drugs in their home environment, whereas cocaine was more rewarding than heroin outside the home. All measures of drug reward (intake, motivation, affect) were affected in a similar manner (Caprioli et al. 2007a, 2008; Celentano et al. 2009; Avvisati et al. 2016). Furthermore, when given the choice between heroin and cocaine the rats preferred heroin to cocaine in the home environment and cocaine to heroin outside the home (Caprioli et al. 2009). Finally, the ability of a priming dose of heroin or cocaine to precipitate drug seeking in animal model of relapse was also affected by the setting (Montanari et al. 2015).

Translational studies conducted in addicts (Caprioli et al. 2009; Badiani and Spagnolo 2013) have shown that even in humans drug and setting interact in a manner similar to that observed in rats, even if the experimental design was necessarily different. Heroin and cocaine co-abusers reported in fact to use heroin prevalently at home and cocaine prevalently outside the home.

**4.3. An emotional appraisal hypothesis of drug reward**

The substance-specificity of the modulatory influence of setting on drug reward was at least initially (Caprioli et al. 2007a, 2008) an unexpected finding. Indeed, the results described in the previous sections were at odds with the prevalent conception of drug reward as a unitary phenomenon and could not be easily accommodated in any extant theoretical model. To account for these findings, Badiani has proposed a novel model of drug reward, according to which the setting may influence drug reward "by acting as an ecological backdrop for the

appraisal of drug effects." (Badiani 2013). In particular, Badiani hypothesized that each addictive drug produces a distinctive spectra of central and peripheral effects that do not always 'match' the setting where the drug is used. Cocaine, for example, produces a state of central arousal and sympathomimetic effects that would be perceived as at odds with a familiar home environment. In contrast, the sedative effects of heroin would be at odds with dangerous non-domestic environments. In summary, according to this model, the overall rewarding effect of a drug is thwarted in the presence of a mismatch between its central and peripheral effects and the setting of drug taking.

A number of predictions derived from the hypothesis highlighted above can be subjected to experimental verification in animals and humans. This has led me to conduct the experiments outlined in Section 5.

## 5. Aims of the present dissertation

My dissertation aims at testing two predictions derived from the hypothesis put forward by Badiani (2013) and summarized in Section 4.4. The first prediction is that the affective state produced by cocaine and heroin may undergo a change in valence as a function of the setting. In particular, Badiani's hypothesis predicts that the affective valence of heroin is more positive when the drug is taken at home than when is taken outside the home. The opposite should occur for cocaine. Initial support for this has been provided by an animal study, already described in Section 4.2.2, in which 50 kHz USVs were used to assess the emotional valence of drug experience (Avvisati et al. 2016). In Chapter 2, I will report on a study designed to investigate this prediction in

human addicts using a two-dimensional model of affect (Russell 1980). This model posits that affective states arise from two independent neurophysiological systems related to arousal (a low-high energy continuum) and to valence (a pleasure-displeasure continuum). The main advantage of this model is that it allowed us to test the more specific prediction of a shift in the affective valence of heroin in the individuals who experienced its sedative effect. The same line reasoning applied to the activating effects of cocaine.

Another prediction derived from Badiani's hypothesis was that the setting might alter in opposite directions the effects of heroin and cocaine in at least some of the brain areas implicated in brain reward. In Chapter 3, I will report on a study in which we used emotional imagery and 3T functional Magnetic Resonance Imaging (fMRI) to investigate drug and setting interaction in addicts with a history of heroin and cocaine abuse. We adapted an emotional imagery procedure based on previous work by Lang and colleagues (1979, 1993) to recreate two different real-world settings of drug use and asked the participants to imagine taking heroin and cocaine. We hypothesized a double dissociation of the BOLD signal in the brain regions implicated in drug reward, such as the prefrontal cortex and the striatum.

## 6. References


Amara, S. G., and M. J. Kuhar. 1993. "Neurotransmitter Transporters: Recent Progress." *Annual Review of Neuroscience* 16: 73–93.

Anderson, Carl M., Luis C. Maas, Blaise Deb Frederick, Jacob T. Bendor, Thomas J. Spencer, Eli Livni, Scott E. Lukas, et al. 2006. "Cerebellar Vermis Involvement in Cocaine-Related Behaviors." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 31 (6): 1318–26.

Antonilli, Letizia, Federico Semeraro, Carmen Suriano, Luciano Signore, and Paolo Nencini. 2003. "High Levels of Morphine-6-Glucuronide in Street Heroin Addicts." *Psychopharmacology* 170 (2): 200–204.

Arbuthnott, Gordon W., and Jeff Wickens. 2007. "Space, Time and Dopamine." *Trends in Neurosciences* 30 (2): 62–69.

Avvisati, Riccardo, Laura Contu, Emiliana Stendardo, Caterina Michetti, Christian Montanari, Maria Luisa Scattoni, and Aldo Badiani. 2016. "Ultrasonic Vocalization in Rats Self-Administering Heroin and Cocaine in Different Settings: Evidence of Substance-Specific Interactions between Drug and Setting." *Psychopharmacology* 233 (8): 1501–11.

Badiani, A., S. G. Anagnostaras, and T. E. Robinson. 1995. "The Development of Sensitization to the Psychomotor Stimulant Effects of Amphetamine Is Enhanced in a Novel Environment." *Psychopharmacology* 117 (4): 443–52.

Badiani, A., K. E. Browman, and T. E. Robinson. 1995. "Influence of Novel versus Home Environments on Sensitization to the Psychomotor Stimulant Effects of Cocaine and Amphetamine." *Brain Research* 674 (2): 291–98.

Badiani, A., D. M. Camp, and T. E. Robinson. 1997. "Enduring Enhancement of Amphetamine Sensitization by Drug-Associated Environmental Stimuli." *The Journal of Pharmacology and Experimental Therapeutics* 282 (2): 787–94.

Badiani, Aldo. 2013. "Substance-Specific Environmental Influences on Drug Use and Drug Preference in Animals and Humans." *Current Opinion in Neurobiology* 23 (4): 588–96.

Badiani Aldo. 2014. "Is a 'General' Theory of Addiction Possible? A Commentary on: A Multistep General Theory of Transition to Addiction." *Psychopharmacology* 231 (19): 3923–27.

Badiani, Aldo, David Belin, David Epstein, Donna Calu, and Yavin Shaham. 2011. "Opiate versus Psychostimulant Addiction: The Differences Do Matter." *Nature Reviews. Neuroscience* 12 (11): 685–700.

Badiani, Aldo, Kent C. Berridge, Markus Heilig, David J. Nutt, and Terry E. Robinson. 2017. "Addiction Research and Theory: A Commentary on the Surgeon General's Report on Alcohol, Drugs, and Health." *Addiction Biology*, February. doi:10.1111/adb.12497.

Badiani, Aldo, and Primavera A. Spagnolo. 2013. "Role of Environmental Factors in Cocaine Addiction." *Current Pharmaceutical Design* 19 (40): 6996–7008.

Badiani, A., M. I. Morano, H. Akil, and T. E. Robinson. 1995. "Circulating Adrenal Hormones Are Not Necessary for the Development of Sensitization to the Psychomotor Activating Effects of Amphetamine (Brain Res. 673 (1995) 13--24)(BRES 10891)." *Brain Research* 678 (1). Elsevier: i.

Badiani, A., M. M. Oates, H. E. Day, S. J. Watson, H. Akil, and T. E. Robinson. 1998. "Amphetamine-Induced Behavior, Dopamine Release, and c-Fos mRNA Expression: Modulation by Environmental Novelty." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 18 (24): 10579–93.

Badiani, A; Oates, M M; Day, H E; Watson, S J; Akil, H; Robinson, T E. 1999. "Environmental Modulation of Amphetamine-Induced c-Fos Expression in D1 versus D2 Striatal Neurons." *Behavioural Brain Research* 103 (2): 203–9.

Badiani, A., M. M. Oates, S. Fraioli, K. E. Browman, M. M. Ostrander, C. J. Xue, M. E. Wolf, and T. E. Robinson. 2000. "Environmental Modulation of the Response to Amphetamine: Dissociation between Changes in Behavior and Changes in Dopamine and Glutamate Overflow in the Rat Striatal Complex." *Psychopharmacology* 151 (2-3): 166–74.

Badiani, A., M. M. Oates, and T. E. Robinson. 2000. "Modulation of Morphine Sensitization in the Rat by Contextual Stimuli." *Psychopharmacology* 151 (2-3): 273–82.

Badiani, A., and T. E. Robinson. 2004. "Drug-Induced Neurobehavioral Plasticity: The Role of Environmental Context." *Behavioural Pharmacology* 15 (5-6): 327–39.

Balleine, B. W., and A. Dickinson. 1998. "Goal-Directed Instrumental Action: Contingency and Incentive Learning and Their Cortical Substrates." *Neuropharmacology* 37 (4-5): 407–19.

Balsters, Joshua H., Christopher D. Whelan, Ian H. Robertson, and Narender Ramnani. 2013. "Cerebellum and Cognition: Evidence for the Encoding of Higher Order Rules." *Cerebral Cortex* 23 (6): 1433–43.

Barker, David J., David H. Root, Sisi Ma, Shaili Jha, Laura Megehee, Anthony P. Pawlak, and Mark O. West. 2010. "Dose-Dependent Differences in Short Ultrasonic Vocalizations Emitted by Rats during Cocaine Self-Administration." *Psychopharmacology* 211 (4): 435–42.

Baumann, Oliver, and Jason B. Mattingley. 2012. "Functional Topography of Primary Emotion Processing in the Human Cerebellum." *NeuroImage* 61 (4): 805–11.

Bausch, S. B., T. A. Patterson, M. U. Ehrengruber, H. A. Lester, N. Davidson, and C. Chavkin. 1995. "Colocalization of Mu Opioid Receptors with GIRK1 Potassium Channels in the Rat Brain: An Immunocytochemical Study." *Receptors & Channels* 3 (3): 221–41.

Beaulieu, Jean-Martin, Stefano Espinoza, and Raul R. Gainetdinov. 2015. "Dopamine Receptors - IUPHAR Review 13." *British Journal of Pharmacology* 172 (1): 1–23.

Berridge, Kent C. 2004. "Motivation Concepts in Behavioral Neuroscience." *Physiology & Behavior* 81 (2): 179–209.

Berridge, Kent C. 2007. "The Debate over Dopamine's Role in Reward: The Case for Incentive Salience." *Psychopharmacology* 191 (3): 391–431.

Berridge, Kent C. 2012. "From Prediction Error to Incentive Salience: Mesolimbic Computation of Reward Motivation." *The European Journal of Neuroscience* 35 (7): 1124–43.

Berridge, Kent C., and Morten L. Kringelbach. 2013. "Neuroscience of Affect: Brain Mechanisms of Pleasure and Displeasure." *Current Opinion in Neurobiology* 23 (3): 294–303.

Berridge, Kent C., Terry E. Robinson, and J. Wayne Aldridge. 2009. "Dissecting Components of Reward: 'Liking', 'Wanting', and Learning." *Current Opinion in Pharmacology* 9 (1): 65–73.

Beuming, Thijs, Julie Kniazeff, Marianne L. Bergmann, Lei Shi, Luis Gracia, Klaudia Raniszewska, Amy Hauck Newman, et al. 2008. "The Binding Sites for Cocaine and Dopamine in the Dopamine Transporter Overlap." *Nature Neuroscience* 11 (7): 780–89.

Bilder, Robert M., Jan Volavka, Herbert M. Lachman, and Anthony A. Grace. 2004. "The Catechol-O-Methyltransferase Polymorphism: Relations to the Tonic-Phasic Dopamine Hypothesis and Neuropsychiatric Phenotypes." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 29 (11): 1943–61.

Bindra, D. 1974. "A Motivational View of Learning, Performance, and Behavior Modification." *Psychological Review* 81 (3): 199–213.

Bindra, Dalbir. 1959. *Motivation: A Systematic Reinterpretation*. John Wiley & Sons Canada, Limited.

Blakely, R. D., L. J. De Felice, and H. C. Hartzell. 1994. "Molecular Physiology of Norepinephrine and Serotonin Transporters." *The Journal of Experimental Biology* 196 (November): 263–81.

Boileau, Isabelle, Alain Dagher, Marco Leyton, Roger N. Gunn, Glen B. Baker, Mirko Diksic, and Chawki Benkelfat. 2006. "Modeling Sensitization to Stimulants in Humans: An [11C]raclopride/positron Emission Tomography Study in Healthy Men." *Archives of General Psychiatry* 63 (12): 1386–95.

Bonson, Katherine R., Steven J. Grant, Carlo S. Contoreggi, Jonathan M. Links, Janet Metcalfe, H. Lloyd Weyl, Varughese Kurian, Monique Ernst, and Edythe D. London. 2002. "Neural Systems and Cue-Induced Cocaine Craving." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 26 (3): 376–86.

Breier, A., T. P. Su, and R. Saunders. 1997. "Schizophrenia Is Associated with Elevated Amphetamine-Induced Synaptic Dopamine Concentrations: Evidence from a Novel Positron Emission Tomography Method." *Proceedings of the*. National Acad Sciences. http://www.pnas.org/content/94/6/2569.short.

Browman, K. E., A. Badiani, and T. E. Robinson. 1998a. "The Influence of Environment on the Induction of Sensitization to the Psychomotor Activating Effects of Intravenous Cocaine in Rats Is Dose-Dependent." *Psychopharmacology* 137 (1): 90–98.

Browman, K E; Badiani, A; Robinson, T E. 1998b. "Modulatory Effect of Environmental Stimuli on the Susceptibility to Amphetamine Sensitization: A Dose-Effect Study in Rats." *The Journal of Pharmacology and Experimental Therapeutics* 287 (3): 1007–14.

Brown, G. P., K. Yang, M. A. King, G. C. Rossi, L. Leventhal, A. Chang, and G. W. Pasternak. 1997. "3-Methoxynaltrexone, a Selective Heroin/morphine-6beta-Glucuronide Antagonist." *FEBS Letters* 412 (1): 35–38.

Caine, S. Barak, S. Stevens Negus, Nancy K. Mello, Smita Patel, Linda Bristow, Janusz Kulagowski, Daniela Vallone, Adolfo Saiardi, and Emiliana Borrelli. 2002. "Role of Dopamine D2-like Receptors in Cocaine Self-Administration: Studies with D2 Receptor Mutant Mice and Novel D2 Receptor Antagonists." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 22 (7): 2977–88.

Caine, S. Barak, Morgane Thomsen, Kara I. Gabriel, Jill S. Berkowitz, Lisa H. Gold, George F. Koob, Susumu Tonegawa, Jianhua Zhang, and Ming Xu. 2007. "Lack of Self-Administration of Cocaine in Dopamine D1 Receptor Knock-out Mice." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 27 (48): 13140–50.

Caprioli, Daniele, Michele Celentano, Alessandro Dubla, Federica Lucantonio, Paolo Nencini, and Aldo Badiani. 2009. "Ambience and Drug Choice: Cocaine- and Heroin-Taking as a Function of Environmental Context in Humans and Rats." *Biological Psychiatry* 65 (10): 893–99.

Caprioli, Daniele, Michele Celentano, Giovanna Paolone, and Aldo Badiani. 2007. "Modeling the Role of Environment in Addiction." *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 31 (8): 1639–53.

Caprioli, Daniele, Michele Celentano, Giovanna Paolone, Federica Lucantonio, Andrea Bari, Paolo Nencini, and Aldo Badiani. 2008. "Opposite Environmental Regulation of Heroin and Amphetamine Self-Administration in the Rat." *Psychopharmacology* 198 (3): 395–404.

Caprioli, Daniele, Giovanna Paolone, Michele Celentano, Arianna Testa, Paolo Nencini, and Aldo Badiani. 2007. "Environmental Modulation of Cocaine Self-Administration in the Rat." *Psychopharmacology* 192 (3): 397–406.

Caprioli, Daniele, Marco Venniro, Tamara Zeric, Xuan Li, Sweta Adhikary, Rajtarun Madangopal, Nathan J. Marchant, et al. 2015. "Effect of the Novel Positive Allosteric Modulator of Metabotropic Glutamate Receptor 2 AZD8529 on Incubation of Methamphetamine Craving After Prolonged Voluntary Abstinence in a Rat Model." *Biological Psychiatry* 78 (7): 463–73.

Celentano, Michele, Daniele Caprioli, Pasqua Dipasquale, Pasqua Di Pasquale, Veronica Cardillo, Paolo Nencini, Silvana Gaetani, and Aldo Badiani. 2009. "Drug Context Differently Regulates Cocaine versus Heroin Self-Administration and Cocaine- versus Heroin-Induced Fos mRNA Expression in the Rat." *Psychopharmacology* 204 (2): 349–60.

Chefer, Vladimir I., Brigitte L. Kieffer, and Toni S. Shippenberg. 2003. "Basal and Morphine-Evoked Dopaminergic Neurotransmission in the Nucleus Accumbens of MOR-and DOR-Knockout Mice." *The European Journal of Neuroscience* 18 (7). Wiley Online Library: 1915–22.

Christrup, L. L. 1997. "Morphine Metabolites." *Acta Anaesthesiologica Scandinavica* 41 (1 Pt 2): 116–22.

Cohen, Jeremiah Y., Sebastian Haesler, Linh Vong, Bradford B. Lowell, and Naoshige Uchida. 2012. "Neuron-Type-Specific Signals for Reward and Punishment in the Ventral Tegmental Area." *Nature* 482 (7383): 85–88.

Covey, Dan P., Mitchell F. Roitman, and Paul A. Garris. 2014. "Illicit Dopamine Transients: Reconciling Actions of Abused Drugs." *Trends in Neurosciences* 37 (4): 200–210.

Cox, Sylvia M. L., Chawki Benkelfat, Alain Dagher, J. Scott Delaney, France Durand, Theodore Kolivakis, Kevin F. Casey, and Marco Leyton. 2011. "Effects of Lowered Serotonin Transmission on Cocaine-Induced Striatal Dopamine Response: PET [$^{11}$C]raclopride Study in Humans." *The British Journal of Psychiatry: The Journal of Mental Science* 199 (5): 391–97.

Crombag, H. S., A. Badiani, J. Chan, J. Dell'Orco, S. P. Dineen, and T. E. Robinson. 2001. "The Ability of Environmental Context to Facilitate Psychomotor Sensitization to Amphetamine Can Be Dissociated from Its Effect on Acute Drug Responsiveness and on Conditioned Responding." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 24 (6): 680–90.

Crombag, H. S., A. Badiani, S. Maren, and T. E. Robinson. 2000. "The Role of Contextual versus Discrete Drug-Associated Cues in Promoting the Induction of Psychomotor Sensitization to Intravenous Amphetamine." *Behavioural Brain Research* 116 (1): 1–22.

Crombag, H. S., A. Badiani, and T. E. Robinson. 1996. "Signalled versus Unsignalled Intravenous Amphetamine: Large Differences in the Acute Psychomotor Response and Sensitization." *Brain Research* 722 (1-2): 227–31.

Daglish, Mark R. C., Tim M. Williams, Sue J. Wilson, Lindsay G. Taylor, Chin B. Eap, Marc Augsburger, Christian Giroud, et al. 2008. "Brain Dopamine Response in Human Opioid Addiction." *The British Journal of Psychiatry: The Journal of Mental Science* 193 (1): 65–72.

Darvas, Martin, and Richard D. Palmiter. 2010. "Restricting Dopaminergic Signaling to Either Dorsolateral or Medial Striatum Facilitates Cognition." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 30 (3): 1158–65.

Day, H. E., A. Badiani, J. M. Uslaner, M. M. Oates, N. M. Vittoz, T. E. Robinson, S. J. Watson Jr, and H. Akil. 2001. "Environmental Novelty Differentially Affects c-Fos mRNA Expression Induced by Amphetamine or Cocaine in Subregions of the Bed Nucleus of the Stria Terminalis and Amygdala." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 21 (2): 732–40.

Devine, D. P., P. Leone, D. Pocock, and R. A. Wise. 1993. "Differential Involvement of Ventral Tegmental Mu, Delta and Kappa Opioid Receptors in Modulation of Basal Mesolimbic Dopamine Release: In Vivo Microdialysis Studies." *The Journal of Pharmacology and Experimental Therapeutics* 266 (3): 1236–46.

Di Chiara, G., and A. Imperato. 1986. "Preferential Stimulation of Dopamine Release in the Nucleus Accumbens by Opiates, Alcohol, and Barbiturates: Studies with Transcerebral Dialysis in Freely Moving Rats." *Annals of the New York Academy of Sciences* 473: 367–81.

Di Chiara, G., P. Loddo, and G. Tanda. 1999. "Reciprocal Changes in Prefrontal and Limbic Dopamine Responsiveness to Aversive and Rewarding Stimuli after Chronic Mild Stress: Implications for the Psychobiology of Depression." *Biological Psychiatry* 46 (12): 1624–33.

Durieux, Pierre F., Bertrand Bearzatto, Stefania Guiducci, Thorsten Buch, Ari Waisman, Michele Zoli, Serge N. Schiffmann, and Alban de Kerchove d'Exaerde. 2009. "D2R

Striatopallidal Neurons Inhibit Both Locomotor and Drug Reward Processes." *Nature Neuroscience* 12 (4): 393–95.

Dworkin, S. I., G. F. Guerin, C. Co, N. E. Goeders, and J. E. Smith. 1988. "Lack of an Effect of 6-Hydroxydopamine Lesions of the Nucleus Accumbens on Intravenous Morphine Self-Administration." *Pharmacology, Biochemistry, and Behavior* 30 (4): 1051–57.

Eddy, N. B., H. Halbach, and O. J. Braenden. 1957. "Synthetic Substances with Morphine-like Effect: Clinical Experience; Potency, Side-Effects, Addiction Liability." *Bulletin of the World Health Organization* 17 (4-5): 569–863.

Ettenberg, A., H. O. Pettit, F. E. Bloom, and G. F. Koob. 1982. "Heroin and Cocaine Intravenous Self-Administration in Rats: Mediation by Separate Neural Systems." *Psychopharmacology* 78 (3): 204–9.

Faura, C. C., S. L. Collins, R. A. Moore, and H. J. McQuay. 1998. "Systematic Review of Factors Affecting the Ratios of Morphine and Its Major Metabolites." *Pain* 74 (1): 43–53.

Ferguson, Susan M., Camille S. Norton, Stanley J. Watson, Huda Akil, and Terry E. Robinson. 2003. "Amphetamine-Evoked c-Fos mRNA Expression in the Caudate-Putamen: The Effects of DA and NMDA Receptor Antagonists Vary as a Function of Neuronal Phenotype and Environmental Context." *Journal of Neurochemistry* 86 (1): 33–44.

Ferguson, Susan M., Mark J. Thomas, and Terry E. Robinson. 2004. "Morphine-Induced c-Fos mRNA Expression in Striatofugal Circuits: Modulation by Dose, Environmental Context, and Drug History." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 29 (9): 1664–74.

Fields, Howard L., and Elyssa B. Margolis. 2015. "Understanding Opioid Reward." *Trends in Neurosciences* 38 (4): 217–25.

Fiorino, D. F., A. Coury, and A. G. Phillips. 1997. "Dynamic Changes in Nucleus Accumbens Dopamine Efflux during the Coolidge Effect in Male Rats." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 17 (12): 4849–55.

Floresco, Stan B., Anthony R. West, Brian Ash, Holly Moore, and Anthony A. Grace. 2003. "Afferent Modulation of Dopamine Neuron Firing Differentially Regulates Tonic and Phasic Dopamine Transmission." *Nature Neuroscience* 6 (9): 968–73.

Fraioli, S., H. S. Crombag, A. Badiani, and T. E. Robinson. 1999. "Susceptibility to Amphetamine-Induced Locomotor Sensitization Is Modulated by Environmental Stimuli." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 20 (6): 533–41.

Fuente-Fernández, Raúl de la, Anthony G. Phillips, Mariangela Zamburlini, Vesna Sossi, Donald B. Calne, Thomas J. Ruth, and A. Jon Stoessl. 2002. "Dopamine Release in Human Ventral Striatum and Expectation of Reward." *Behavioural Brain Research* 136 (2): 359–63.

Garzón, M., and V. M. Pickel. 2001. "Plasmalemmal Mu-Opioid Receptor Distribution Mainly in Nondopaminergic Neurons in the Rat Ventral Tegmental Area." *Synapse* 41 (4): 311–28.

Georges, F., and G. Aston-Jones. 2001. "Potent Regulation of Midbrain Dopamine Neurons by the Bed Nucleus of the Stria Terminalis." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 21 (16): RC160.

Gerrits, M. A., and J. M. Van Ree. 1996. "Effect of Nucleus Accumbens Dopamine Depletion on Motivational Aspects Involved in Initiation of Cocaine and Heroin Self-Administration in Rats." *Brain Research* 713 (1-2): 114–24.

Grace, Anthony A. 2016. "Dysregulation of the Dopamine System in the Pathophysiology of Schizophrenia and Depression." *Nature Reviews. Neuroscience* 17 (8): 524–32.

Grant, S., E. D. London, D. B. Newlin, V. L. Villemagne, X. Liu, C. Contoreggi, R. L. Phillips, A. S. Kimes, and A. Margolin. 1996. "Activation of Memory Circuits during Cue-Elicited Cocaine Craving." *Proceedings of the National Academy of Sciences of the United States of America* 93 (21): 12040–45.

Greenwell, Thomas N., Abraham Zangen, Sheryl Martin-Schild, Roy A. Wise, and James E. Zadina. 2002. "Endomorphin-1 and-2 Immunoreactive Cells in the Hypothalamus Are Labeled by Fluoro-Gold Injections to the Ventral Tegmental Area." *The Journal of Comparative Neurology* 454 (3). Wiley Online Library: 320–28.

Gutstein, Howard B., Huda Akil, and Others. 2001. "Opioid Analgesics." *Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th Ed. Edited by Hardman JG, Limbird LE, Gilman AG. New York, McGraw-Hill*, 569–620.

Gysling, K., and R. Y. Wang. 1983. "Morphine-Induced Activation of A10 Dopamine Neurons in the Rat." *Brain Research* 277 (1): 119–27.

Haney, Margaret, and Roger Spealman. 2008. "Controversies in Translational Research: Drug Self-Administration." *Psychopharmacology* 199 (3): 403–19.

Holstege, Gert, and Janniko R. Georgiadis. 2004. "The Emotional Brain: Neural Correlates of Cat Sexual Behavior and Human Male Ejaculation." *Progress in Brain Research* 143: 39–45.

Hope, Bruce T., Danielle E. Simmons, Tim B. Mitchell, Justin D. Kreuter, and Brandi J. Mattson. 2006. "Cocaine-Induced Locomotor Activity and Fos Expression in Nucleus Accumbens Are Sensitized for 6 Months after Repeated Cocaine Administration Outside the Home Cage." *The European Journal of Neuroscience* 24 (3): 867–75.

Imperato, A., L. Angelucci, P. Casolini, A. Zocchi, and S. Puglisi-Allegra. 1992. "Repeated Stressful Experiences Differently Affect Limbic Dopamine Release during and Following Stress." *Brain Research* 577 (2): 194–99.

Imperato, A., S. Puglisi-Allegra, P. Casolini, and L. Angelucci. 1991. "Changes in Brain Dopamine and Acetylcholine Release during and Following Stress Are Independent of the Pituitary-Adrenocortical Axis." *Brain Research* 538 (1): 111–17.

Inturrisi, C. E., M. B. Max, K. M. Foley, M. Schultz, S. U. Shin, and R. W. Houde. 1984. "The Pharmacokinetics of Heroin in Patients with Chronic Pain." *The New England Journal of Medicine* 310 (19): 1213–17.

Jalabert, Marion, Romain Bourdy, Julien Courtin, Pierre Veinante, Olivier J. Manzoni, Michel Barrot, and François Georges. 2011. "Neuronal Circuits Underlying Acute Morphine Action on Dopamine Neurons." *Proceedings of the National Academy of Sciences of the United States of America* 108 (39): 16446–50.

Jatlow, P., J. D. Elsworth, C. W. Bradberry, G. Winger, J. R. Taylor, R. Russell, and R. H. Roth. 1991. "Cocaethylene: A Neuropharmacologically Active Metabolite Associated with Concurrent Cocaine-Ethanol Ingestion." *Life Sciences* 48 (18): 1787–94.

Jeffcoat, A. R., M. Perez-Reyes, J. M. Hill, B. M. Sadler, and C. E. Cook. 1989. "Cocaine Disposition in Humans after Intravenous Injection, Nasal Insufflation (snorting), or Smoking." *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 17 (2): 153–59.

Johnson, S. W., and R. A. North. 1992. "Opioids Excite Dopamine Neurons by Hyperpolarization of Local Interneurons." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 12 (2): 483–88.

Jones, R. T. 1990. "The Pharmacology of Cocaine Smoking in Humans." *NIDA Research Monograph* 99: 30–41.

Kendler, Kenneth S., Kristen C. Jacobson, Carol A. Prescott, and Michael C. Neale. 2003. "Specificity of Genetic and Environmental Risk Factors for Use and Abuse/dependence of Cannabis, Cocaine, Hallucinogens, Sedatives, Stimulants, and Opiates in Male Twins." *The American Journal of Psychiatry* 160 (4): 687–95.

Kendler, Kenneth S., John Myers, and Carol A. Prescott. 2007. "Specificity of Genetic and Environmental Risk Factors for Symptoms of Cannabis, Cocaine, Alcohol, Caffeine, and Nicotine Dependence." *Archives of General Psychiatry* 64 (11): 1313–20.

Kishida, Kenneth T., Stefan G. Sandberg, Terry Lohrenz, Youssef G. Comair, Ignacio Sáez, Paul E. M. Phillips, and P. Read Montague. 2011. "Sub-Second Dopamine Detection in Human Striatum." *PloS One* 6 (8): e23291.

Kiyatkin, E. A. 1994. "Behavioral Significance of Phasic Changes in Mesolimbic Dopamine-Dependent Electrochemical Signal Associated with Heroin Self-Injections." *Journal of Neural Transmission. General Section* 96 (3): 197–214.

Kiyatkin, E. A., and G. V. Rebec. 2001. "Impulse Activity of Ventral Tegmental Area Neurons during Heroin Self-Administration in Rats." *Neuroscience* 102 (3): 565–80.

Kiyatkin, Eugene A., and George V. Rebec. 1997. "Activity of Presumed Dopamine Neurons in the Ventral Tegmental Area during Heroin Self-administration." *Neuroreport* 8 (11): 2581.

Knutson, B., J. Burgdorf, and J. Panksepp. 1998. "Anticipation of Play Elicits High-Frequency Ultrasonic Vocalizations in Young Rats." *Journal of Comparative Psychology* 112 (1): 65–73.

Kravitz, Alexxai V., Lynne D. Tye, and Anatol C. Kreitzer. 2012. "Distinct Roles for Direct and Indirect Pathway Striatal Neurons in Reinforcement." *Nature Neuroscience* 15 (6): 816–18.

Lammel, Stephan, Andrea Hetzel, Olga Häckel, Ian Jones, Birgit Liss, and Jochen Roeper. 2008. "Unique Properties of Mesoprefrontal Neurons within a Dual Mesocorticolimbic Dopamine System." *Neuron* 57 (5): 760–73.

Lammel, Stephan, Byung Kook Lim, and Robert C. Malenka. 2014. "Reward and Aversion in a Heterogeneous Midbrain Dopamine System." *Neuropharmacology* 76 Pt B (January): 351–59.

Landry, M. J. 1992. "An Overview of Cocaethylene, an Alcohol-Derived, Psychoactive, Cocaine Metabolite." *Journal of Psychoactive Drugs* 24 (3): 273–76.

Lang, Peter J. 1979. “A Bio-Informational Theory of Emotional Imagery.” *Psychophysiology* 16 (6). Wiley Online Library: 495–512.

Lang, P. J., M. K. Greenwald, M. M. Bradley, and A. O. Hamm. 1993. “Looking at Pictures: Affective, Facial, Visceral, and Behavioral Reactions.” *Psychophysiology* 30 (3): 261–73.

Laruelle, M., A. Abi-Dargham, C. H. van Dyck, W. Rosenblatt, Y. Zea-Ponce, S. S. Zoghbi, R. M. Baldwin, D. S. Charney, P. B. Hoffer, and H. F. Kung. 1995. “SPECT Imaging of Striatal Dopamine Release after Amphetamine Challenge.” *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine* 36 (7): 1182–90.

Lenoir, Magalie, and Serge H. Ahmed. 2008. “Supply of a Nondrug Substitute Reduces Escalated Heroin Consumption.” *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 33 (9): 2272–82.

Leyton, Marco, Isabelle Boileau, Chawki Benkelfat, Mirko Diksic, Glen Baker, and Alain Dagher. 2002. “Amphetamine-Induced Increases in Extracellular Dopamine, Drug Wanting, and Novelty Seeking: A PET/[11C] Raclopride Study in Healthy Men.” *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 27 (6). Nature Publishing Group: 1027.

Leyton, Marco, Alain Dagher, Isabelle Boileau, Kevin Casey, Glen B. Baker, Mirko Diksic, Roger Gunn, Simon N. Young, and Chawki Benkelfat. 2004. “Decreasing Amphetamine-Induced Dopamine Release by Acute Phenylalanine/tyrosine Depletion: A PET/[11C] Raclopride Study in Healthy Men.” *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 29 (2). Nature Publishing Group: 427.

Lodge, D. J., and A. A. Grace. 2006. “The Laterodorsal Tegmentum Is Essential for Burst Firing of Ventral Tegmental Area Dopamine Neurons.” *Proceedings of the National Academy of Sciences of the United States of America* 103 (13): 5167–72.

Mahler, Stephen V., Elena M. Vazey, Jacob T. Beckley, Colby R. Keistler, Ellen M. McGlinchey, Jennifer Kaufling, Steven P. Wilson, Karl Deisseroth, John J. Woodward, and Gary Aston-Jones. 2014. “Designer Receptors Show Role for Ventral Pallidum Input to Ventral Tegmental Area in Cocaine Seeking.” *Nature Neuroscience* 17 (4): 577–85.

Malenka, Robert C., Nestler, Eric J., Hyman, Steven E. 2009. "Chapter 6: Widely Projecting Systems: Monoamines, Acetylcholine, and Orexin". In Sydor A, Brown RY. Molecular Neuropharmacology: A Foundation for Clinical Neuroscience (2nd ed.). New York: McGraw-Hill Medical. pp. 147–148, 154–157.

Manglik, Aashish, Henry Lin, Dipendra K. Aryal, John D. McCorvy, Daniela Dengler, Gregory Corder, Anat Levit, et al. 2016. “Structure-Based Discovery of Opioid Analgesics with Reduced Side Effects.” *Nature* 537 (7619): 185–90.

Marcellino, Daniel, Sergi Ferré, Vicent Casadó, Antonio Cortés, Bernard Le Foll, Carmen Mazzola, Filippo Drago, Et Al. 2008. “Identification Of Dopamine D1–D3 Receptor Heteromers: Indications for a role of synergistic D1-D3 Receptor Interactions in the Striatum” *The Journal Of Biological Chemistry* 283 (38): 26016–25.

Margolis, Elyssa B., Hagar Lock, Gregory O. Hjelmstad, and Howard L. Fields. 2006. “The Ventral Tegmental Area Revisited: Is There an Electrophysiological Marker for Dopaminergic Neurons?” *The Journal of Physiology* 577 (Pt 3): 907–24.

Margolis, Elyssa B., Brian Toy, Patricia Himmels, Marisela Morales, and Howard L. Fields. 2012. "Identification of Rat Ventral Tegmental Area GABAergic Neurons." *PloS One* 7 (7): e42365.

Martin-Sölch, C., S. Magyar, G. Künig, J. Missimer, W. Schultz, and K. L. Leenders. 2001. "Changes in Brain Activation Associated with Reward Processing in Smokers and Nonsmokers. A Positron Emission Tomography Study." *Experimental Brain Research. Experimentelle Hirnforschung. Experimentation Cerebrale* 139 (3): 278–86.

Martin, W. R., and H. F. Fraser. 1961. "A Comparative Study of Physiological and Subjective Effects of Heroin and Morphine Administered Intravenously in Postaddicts." *The Journal of Pharmacology and Experimental Therapeutics* 133 (September): 388–99.

Masson, J., C. Sagné, M. Hamon, and S. El Mestikawy. 1999. "Neurotransmitter Transporters in the Central Nervous System." *Pharmacological Reviews* 51 (3): 439–64.

Matthews, R. T., and D. C. German. 1984. "Electrophysiological Evidence for Excitation of Rat Ventral Tegmental Area Dopamine Neurons by Morphine." *Neuroscience* 11 (3): 617–25.

McGinnis, Marilyn Y., and M. Vakulenko. 2003. "Characterization of 50-kHz Ultrasonic Vocalizations in Male and Female Rats." *Physiology & Behavior* 80 (1): 81–88.

Melis, M., G. L. Gessa, and M. Diana. 2000. "Different Mechanisms for Dopaminergic Excitation Induced by Opiates and Cannabinoids in the Rat Midbrain." *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 24 (6): 993–1006.

Mello, N. K., and S. S. Negus. 1996. "Preclinical Evaluation of Pharmacotherapies for Treatment of Cocaine and Opioid Abuse Using Drug Self-Administration Procedures." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 14 (6): 375–424.

Milne, Robert W., Roger L. Nation, and Andrew A. Somogyi. 1996. "The Disposition of Morphine and Its 3-and 6-Glucuronide Metabolites in Humans and Animals, and the Importance of the Metabolites to the Pharmacological Effects of Morphine." *Drug Metabolism Reviews* 28 (3). Taylor & Francis: 345–472.

Montague, P. R., P. Dayan, and T. J. Sejnowski. 1996. "A Framework for Mesencephalic Dopamine Systems Based on Predictive Hebbian Learning." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 16 (5): 1936–47.

Montanari, Christian, Emiliana Stendardo, Maria Teresa De Luca, Maria Meringolo, Laura Contu, and Aldo Badiani. 2015. "Differential Vulnerability to Relapse into Heroin versus Cocaine-Seeking as a Function of Setting." *Psychopharmacology* 232 (13): 2415–24.

Morris, Genela, Alon Nevet, David Arkadir, Eilon Vaadia, and Hagai Bergman. 2006. "Midbrain Dopamine Neurons Encode Decisions for Future Action." *Nature Neuroscience* 9 (8): 1057–63.

Nieh, Edward H., Sung-Yon Kim, Praneeth Namburi, and Kay M. Tye. 2013. "Optogenetic Dissection of Neural Circuits Underlying Emotional Valence and Motivated Behaviors." *Brain Research* 1511 (May): 73–92.

Norman, Andrew B., Michael R. Tabet, Mantana K. Norman, Brittney K. Fey, Vladimir L. Tsibulsky, and Ronald W. Millard. 2011. "The Affinity of D2-like Dopamine Receptor Antagonists Determines the Time to Maximal Effect on Cocaine Self-Administration." *The Journal of Pharmacology and Experimental Therapeutics* 338 (2): 724–28.

Nutt, David J., Anne Lingford-Hughes, David Erritzoe, and Paul R. A. Stokes. 2015. "The Dopamine Theory of Addiction: 40 Years of Highs and Lows." *Nature Reviews. Neuroscience* 16 (5): 305–12.

Office of the Surgeon General (2016). Facing Addiction in America:TheSurgeon General's Report on Alcohol, Drugs, and Health. Washington, DC: US Dept .of Health and Human Services. http://addiction.surgeongeneral.gov. Accessed November 29, 2016

Olbrich, Hans M., Gabriele Valerius, Christine Paris, Friedemann Hagenbuch, Dieter Ebert, and Freimut D. Juengling. 2006. "Brain Activation during Craving for Alcohol Measured by Positron Emission Tomography." *The Australian and New Zealand Journal of Psychiatry* 40 (2): 171–78.

Ostrander, M. M., A. Badiani, H. E. W. Day, C. S. Norton, S. J. Watson, H. Akil, and T. E. Robinson. 2003. "Environmental Context and Drug History Modulate Amphetamine-Induced c-Fos mRNA Expression in the Basal Ganglia, Central Extended Amygdala, and Associated Limbic Forebrain." *Neuroscience* 120 (2): 551–71.

Paladini, C. A., and J. Roeper. 2014. "Generating Bursts (and Pauses) in the Dopamine Midbrain Neurons." *Neuroscience* 282 (December): 109–21.

Panksepp, J., and J. Burgdorf. 2000. "50-kHz Chirping (laughter?) in Response to Conditioned and Unconditioned Tickle-Induced Reward in Rats: Effects of Social Housing and Genetic Variables." *Behavioural Brain Research* 115 (1): 25–38.

Paolone, Giovanna, Rosetta Burdino, and Aldo Badiani. 2003. "Dissociation in the Modulatory Effects of Environmental Novelty on the Locomotor, Analgesic, and Eating Response to Acute and Repeated Morphine in the Rat." *Psychopharmacology* 166 (2): 146–55.

Paolone, Giovanna, David Conversi, Daniele Caprioli, Paola Del Bianco, Paolo Nencini, Simona Cabib, and Aldo Badiani. 2007. "Modulatory Effect of Environmental Context and Drug History on Heroin-Induced Psychomotor Activity and Fos Protein Expression in the Rat Brain." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 32 (12): 2611–23.

Paton, Joseph J., and Kenway Louie. 2012. "Reward and Punishment Illuminated." *Nature Neuroscience* 15 (6): 807–9.

Penson, R. T., Joel S.P., K. Bakhshi, Clark S.J., R. M. Langford, and M. L. Slevin. 2000. "Randomized Placebo-Controlled Trial of the Activity of the Morphine Glucuronides." *Clinical Pharmacology and Therapeutics* 68 (6): 667–76.

Perez-Reyes, M., A. R. Jeffcoat, M. Myers, K. Sihler, and C. E. Cook. 1994. "Comparison in Humans of the Potency and Pharmacokinetics of Intravenously Injected Cocaethylene and Cocaine." *Psychopharmacology* 116 (4): 428–32.

Peters, Jamie, Tommy Pattij, and Taco J. De Vries. 2013. "Targeting Cocaine versus Heroin Memories: Divergent Roles within Ventromedial Prefrontal Cortex." *Trends in Pharmacological Sciences* 34 (12): 689–95.

Pettit, H. O., A. Ettenberg, F. E. Bloom, and G. F. Koob. 1984. "Destruction of Dopamine in the Nucleus Accumbens Selectively Attenuates Cocaine but Not Heroin Self-Administration in Rats." *Psychopharmacology* 84 (2): 167–73.

Phillips, Paul E. M., Josephine M. Johns, Deborah A. Lubin, Evgeny A. Budygin, Raul R. Gainetdinov, Jeffery A. Lieberman, and R. Mark Wightman. 2003. "Presynaptic Dopaminergic Function Is Largely Unaltered in Mesolimbic and Mesostriatal Terminals of Adult Rats That Were Prenatally Exposed to Cocaine." *Brain Research* 961 (1): 63–72.

Pisanu, Augusta, Daniele Lecca, Valentina Valentini, Amine Bahi, Jean-Luc Dreyer, Fabio Cacciapaglia, Andrea Scifo, Giovanna Piras, Cristina Cadoni, and Gaetano Di Chiara. 2015. "Impairment of Acquisition of Intravenous Cocaine Self-Administration by RNA-Interference of Dopamine D1-Receptors in the Nucleus Accumbens Shell." *Neuropharmacology* 89 (February): 398–411.

Puglisi-Allegra, S., A. Imperato, L. Angelucci, and S. Cabib. 1991. "Acute Stress Induces Time-Dependent Responses in Dopamine Mesolimbic System." *Brain Research* 554 (1-2): 217–22.

Ramnani, N., R. Elliott, B. S. Athwal, and R. E. Passingham. 2004. "Prediction Error for Free Monetary Reward in the Human Prefrontal Cortex." *NeuroImage* 23 (3): 777–86.

Rentsch, K. M., G. A. Kullak-Ublick, C. Reichel, P. J. Meier, and K. Fattinger. 2001. "Arterial and Venous Pharmacokinetics of Intravenous Heroin in Subjects Who Are Addicted to Narcotics." *Clinical Pharmacology and Therapeutics* 70 (3): 237–46.

Reynolds, J. N., B. I. Hyland, and J. R. Wickens. 2001. "A Cellular Mechanism of Reward-Related Learning." *Nature* 413 (6851): 67–70.

Richard, Jocelyn M., Daniel C. Castro, Alexandra G. Difeliceantonio, Mike J. F. Robinson, and Kent C. Berridge. 2013. "Mapping Brain Circuits of Reward and Motivation: In the Footsteps of Ann Kelley." *Neuroscience and Biobehavioral Reviews* 37 (9 Pt A): 1919–31.

Robins, Lee N. 1993. "Vietnam Veterans' Rapid Recovery from Heroin Addiction: A Fluke or Normal Expectation?" *Addiction* 88 (8). Blackwell Publishing Ltd: 1041–54.

Robins, L. N., J. E. Helzer, and D. H. Davis. 1975. "Narcotic Use in Southeast Asia and Afterward. An Interview Study of 898 Vietnam Returnees." *Archives of General Psychiatry* 32 (8): 955–61.

Robinson, T. E., and K. C. Berridge. 1993. "The Neural Basis of Drug Craving: An Incentive-Sensitization Theory of Addiction." *Brain Research. Brain Research Reviews* 18 (3): 247–91.

Robinson, T. E., K. E. Browman, H. S. Crombag, and A. Badiani. 1998. "Modulation of the Induction or Expression of Psychostimulant Sensitization by the Circumstances Surrounding Drug Administration." *Neuroscience and Biobehavioral Reviews* 22 (2): 347–54.

Robinson, Terry E., and Bryan Kolb. 2004. "Structural Plasticity Associated with Exposure to Drugs of Abuse." *Neuropharmacology* 47 Suppl 1: 33–46.

Roesch, Matthew R., Donna J. Calu, and Geoffrey Schoenbaum. 2007. "Dopamine Neurons Encode the Better Option in Rats Deciding between Differently Delayed or Sized Rewards." *Nature Neuroscience* 10 (12): 1615–24.

Roitman, Mitchell F., Garret D. Stuber, Paul E. M. Phillips, R. Mark Wightman, and Regina M. Carelli. 2004. "Dopamine Operates as a Subsecond Modulator of Food Seeking." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 24 (6): 1265–71.

Rook, Elisabeth J., Alwin D. R. Huitema, Wim van den Brink, Jan M. van Ree, and Jos H. Beijnen. 2006. "Population Pharmacokinetics of Heroin and Its Major Metabolites." *Clinical Pharmacokinetics* 45 (4): 401–17.

Rossi GC, Brown GP, Leventhal L, Yang K, Pasternak GW. 1996. "Novel receptor mechanisms for heroin and morphine-6 beta-glucuronide analgesia." *Neuroscience Letters* 216 (1): 1-4.

Rossi, Mark A., Tatyana Sukharnikova, Volodya Y. Hayrapetyan, Lucie Yang, and Henry H. Yin. 2013. "Operant Self-Stimulation of Dopamine Neurons in the Substantia Nigra." *PloS One* 8 (6): e65799.

Rothman, Richard B., and Michael H. Baumann. 2003. "Monoamine Transporters and Psychostimulant Drugs." *European Journal of Pharmacology* 479 (1-3): 23–40.

Russell, James A. 1980. "A Circumplex Model of Affect." *Journal of Personality and Social Psychology* 39 (6): 1161–78.

Russo, Scott J., and Eric J. Nestler. 2013. "The Brain Reward Circuitry in Mood Disorders." *Nature Reviews. Neuroscience* 14 (9): 609–25.

Savica, Rodolfo, and Eduardo E. Benarroch. 2014. "Dopamine Receptor Signaling in the Forebrain: Recent Insights and Clinical Implications." *Neurology* 83 (8): 758–67.

Schlaepfer, T. E., G. D. Pearlson, D. F. Wong, S. Marenco, and R. F. Dannals. 1997. "PET Study of Competition between Intravenous Cocaine and [11C]raclopride at Dopamine Receptors in Human Subjects." *The American Journal of Psychiatry* 154 (9): 1209–13.

Schmahmann, Jeremy D. 2010. "The Role of the Cerebellum in Cognition and Emotion: Personal Reflections since 1982 on the Dysmetria of Thought Hypothesis, and Its Historical Evolution from Theory to Therapy." *Neuropsychology Review* 20 (3): 236–60.

Schultz, W., P. Dayan, and P. R. Montague. 1997. "A Neural Substrate of Prediction and Reward." *Science* 275 (5306): 1593–99.

Schultz, Wolfram. 2006. "Behavioral Theories and the Neurophysiology of Reward." *Annual Review of Psychology* 57: 87–115.

Schultz, Wolfram. 2015. "Neuronal Reward and Decision Signals: From Theories to Data." *Physiological Reviews* 95 (3): 853–951.

Sesack, S. R., and V. M. Pickel. 1992. "Dual Ultrastructural Localization of Enkephalin and Tyrosine Hydroxylase Immunoreactivity in the Rat Ventral Tegmental Area: Multiple Substrates for Opiate-Dopamine Interactions." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 12 (4): 1335–50.

Shaham, Yavin, Uri Shalev, Lin Lu, Harriet De Wit, and Jane Stewart. 2003. "The Reinstatement Model of Drug Relapse: History, Methodology and Major Findings." *Psychopharmacology* 168 (1-2): 3–20.

Sholar, M. B., J. H. Mendelson, N. K. Mello, A. J. Siegel, M. J. Kaufman, J. M. Levin, P. F. Renshaw, and B. M. Cohen. 1998. "Concurrent Pharmacokinetic Analysis of Plasma Cocaine and Adrenocorticotropic Hormone in Men." *The Journal of Clinical Endocrinology and Metabolism* 83 (3): 966–68.

Spanagel, R., A. Herz, and T. S. Shippenberg. 1992. "Opposing Tonically Active Endogenous Opioid Systems Modulate the Mesolimbic Dopaminergic Pathway." *Proceedings of the National Academy of Sciences of the United States of America* 89 (6): 2046–50.

Stein, Christoph. 2016. "Opioid Receptors." *Annual Review of Medicine* 67: 433–51.

Stewart, J., and A. Badiani. 1993. "Tolerance and Sensitization to the Behavioral Effects of Drugs." *Behavioural Pharmacology* 4 (4): 289–312.

Toates, Frederick M. 1986. *Motivational Systems*. Cambridge University Press.

Tsuang, M. T., J. L. Bar, R. M. Harley, and M. J. Lyons. 2001. "The Harvard Twin Study of Substance Abuse: What We Have Learned." *Harvard Review of Psychiatry* 9 (6): 267–79.

Tsuang, M. T., M. J. Lyons, R. M. Harley, H. Xian, S. Eisen, J. Goldberg, W. R. True, and S. V. Faraone. 1999. "Genetic and Environmental Influences on Transitions in Drug Use." *Behavior Genetics* 29 (6): 473–79.

Tsuang, M. T., M. J. Lyons, J. M. Meyer, T. Doyle, S. A. Eisen, J. Goldberg, W. True, N. Lin, R. Toomey, and L. Eaves. 1998. "Co-Occurrence of Abuse of Different Drugs in Men: The Role of Drug-Specific and Shared Vulnerabilities." *Archives of General Psychiatry* 55 (11): 967–72.

Uhl, G. R., and P. S. Johnson. 1994. "Neurotransmitter Transporters: Three Important Gene Families for Neuronal Function." *The Journal of Experimental Biology* 196 (November): 229–36.

Ulens, C., L. Baker, A. Ratka, D. Waumans, and J. Tytgat. 2001. "Morphine-6beta-Glucuronide and Morphine-3-Glucuronide, Opioid Receptor Agonists with Different Potencies." *Biochemical Pharmacology* 62 (9): 1273–82.

Uslaner, J., A. Badiani, H. E. Day, S. J. Watson, H. Akil, and T. E. Robinson. 2001. "Environmental Context Modulates the Ability of Cocaine and Amphetamine to Induce c-Fos mRNA Expression in the Neocortex, Caudate Nucleus, and Nucleus Accumbens." *Brain Research* 920 (1-2): 106–16.

Uslaner, J., A. Badiani, C. S. Norton, H. E. Day, S. J. Watson, H. Akil, and T. E. Robinson. 2001. "Amphetamine and Cocaine Induce Different Patterns of c-Fos mRNA Expression in the Striatum and Subthalamic Nucleus Depending on Environmental Context." *The European Journal of Neuroscience* 13 (10): 1977–83.

Volkow, N. D., D. Tomasi, G-J Wang, J. Logan, D. L. Alexoff, M. Jayne, J. S. Fowler, C. Wong, P. Yin, and C. Du. 2014. "Stimulant-Induced Dopamine Increases Are Markedly Blunted in Active Cocaine Abusers." *Molecular Psychiatry* 19 (9): 1037–43.

Volkow, N. D., G. Wang, J. S. Fowler, J. Logan, M. Gerasimov, L. Maynard, Y. Ding, S. J. Gatley, A. Gifford, and D. Franceschi. 2001. "Therapeutic Doses of Oral

Methylphenidate Significantly Increase Extracellular Dopamine in the Human Brain." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 21 (2): RC121.

Volkow, N. D., G. J. Wang, J. S. Fowler, J. Logan, S. J. Gatley, C. Wong, R. Hitzemann, and N. R. Pappas. 1999. "Reinforcing Effects of Psychostimulants in Humans Are Associated with Increases in Brain Dopamine and Occupancy of D(2) Receptors." *The Journal of Pharmacology and Experimental Therapeutics* 291 (1): 409–15.

Volkow, Nora D., and Marisela Morales. 2015. "The Brain on Drugs: From Reward to Addiction." *Cell* 162 (4): 712–25.

Volkow, Nora D., Gene-Jack Wang, Joanna S. Fowler, and Dardo Tomasi. 2012. "Addiction Circuitry in the Human Brain." *Annual Review of Pharmacology and Toxicology* 52: 321–36.

Volkow, Nora D., Gene-Jack Wang, Frank Telang, Joanna S. Fowler, Jean Logan, Anna-Rose Childress, Millard Jayne, Yeming Ma, and Christopher Wong. 2008. "Dopamine Increases in Striatum Do Not Elicit Craving in Cocaine Abusers Unless They Are Coupled with Cocaine Cues." *NeuroImage* 39 (3): 1266–73.

Wagner, B. K., and D. A. O'Hara. 1997. "Pharmacokinetics and Pharmacodynamics of Sedatives and Analgesics in the Treatment of Agitated Critically Ill Patients." *Clinical Pharmacokinetics* 33 (6): 426–53.

Wanat, Matthew J., Ingo Willuhn, Jeremy J. Clark, and Paul E. M. Phillips. 2009. "Phasic Dopamine Release in Appetitive Behaviors and Drug Addiction." *Current Drug Abuse Reviews* 2 (2): 195–213.

Watson, Ben J., Lindsay G. Taylor, Alastair G. Reid, Sue J. Wilson, Paul R. Stokes, David J. Brooks, James F. Myers, Federico E. Turkheimer, David J. Nutt, and Anne R. Lingford-Hughes. 2014a. "Investigating Expectation and Reward in Human Opioid Addiction with [11C] Raclopride PET." *Addiction Biology* 19 (6). Wiley Online Library: 1032–40.

Watson, Ben J.; Taylor, Lindsay G; Reid, Alastair G; Wilson, Sue J; Stokes, Paul R; Brooks, David J; Myers, James F; Turkheimer, Federico E; Nutt, David J; Lingford-Hughes, Anne R 2014b. "Investigating Expectation and Reward in Human Opioid Addiction with [(11) C]raclopride PET." *Addiction Biology* 19 (6): 1032–40.

Welter, Marc, Daniela Vallone, Tarek A. Samad, Hamid Meziane, Alessandro Usiello, and Emiliana Borrelli. 2007. "Absence of Dopamine D2 Receptors Unmasks an Inhibitory Control over the Brain Circuitries Activated by Cocaine." *Proceedings of the National Academy of Sciences of the United States of America* 104 (16): 6840–45.

White, N. R., R. Cagiano, A. U. Moises, and R. J. Barfield. 1990. "Changes in Mating Vocalizations over the Ejaculatory Series in Rats (Rattus Norvegicus)." *Journal of Comparative Psychology* 104 (3): 255–62.

Wise, R. A. 1980. "Action of Drugs of Abuse on Brain Reward Systems." *Pharmacology, Biochemistry, and Behavior* 13 Suppl 1: 213–23.

Wise, Roy A. 2008. "Dopamine and Reward: The Anhedonia Hypothesis 30 Years on." *Neurotoxicity Research* 14 (2-3): 169–83.

Wise, Roy A. 2009. "Roles for Nigrostriatal--Not Just Mesocorticolimbic--Dopamine in Reward and Addiction." *Trends in Neurosciences* 32 (10): 517–24.

Witten, Ilana B., Elizabeth E. Steinberg, Soo Yeun Lee, Thomas J. Davidson, Kelly A. Zalocusky, Matthew Brodsky, Ofer Yizhar, et al. 2011. "Recombinase-Driver Rat Lines: Tools, Techniques, and Optogenetic Application to Dopamine-Mediated Reinforcement." *Neuron* 72 (5): 721–33.

Yeo, Giles S. H., and Lora K. Heisler. 2012. "Unraveling the Brain Regulation of Appetite: Lessons from Genetics." *Nature Neuroscience* 15 (10): 1343–49.

Yoshida, M., H. Yokoo, T. Tanaka, K. Mizoguchi, and H. Emoto. 1993. "Facilitatory Modulation of Mesolimbic Dopamine Neuronal Activity by a μ-Opioid Agonist and Nicotine as Examined with in Vivo Microdialysis." *Brain Research*. Elsevier. http://www.sciencedirect.com/science/article/pii/0006899393900874.

Zaghloul, Kareem A., Justin A. Blanco, Christoph T. Weidemann, Kathryn McGill, Jurg L. Jaggi, Gordon H. Baltuch, and Michael J. Kahana. 2009. "Human Substantia Nigra Neurons Encode Unexpected Financial Rewards." *Science* 323 (5920): 1496–99.

Zhou, Mingyan, Karen Engel, and Joanne Wang. 2007. "Evidence for Significant Contribution of a Newly Identified Monoamine Transporter (PMAT) to Serotonin Uptake in the Human Brain." *Biochemical Pharmacology* 73 (1): 147–54.

Zinberg, Norman E. 1984. "Drug, Set and Setting." *New Haven: Yale University*.

# Chapter 2

## Affective valence of heroin versus cocaine reward in human addicts: the role of setting

## 1. Introduction

### 1.1. Background

As discussed in Chapter 1, retrospective studies in human addicts have shown that the preferred setting of use is not same for all drugs. Indeed, when heroin and cocaine co-abusers were asked about the circumstances of drug taking, they indicated distinct settings for the two drugs: heroin being used preferentially at home and cocaine preferentially outside the home (Caprioli et al. 2009; Badiani and Spagnolo 2013). The fact that similar findings were obtained also in rats (for a review see Badiani 2013) suggests that these preferences were not a trivial consequence of the "addicts' conscious decision to take a sedative drug in a place where one can 'slouch on a sofa', and an activating drug where one can […] move around", but reflected a more fundamental and substance-specific influence of setting on drug reward.

The literature offers many examples of environmental influences on drug taking, including stress and CSs (for a review see Caprioli et al. 2007b). However, there is no theoretical or experimental reason to think that any of these environmental factors should act in a substance-specific manner, and even less to the point of modulating in opposite direction the rewarding effects of two prototypical addictive drugs. Furthermore, extant theoretical frameworks

of drug reward and drug addiction are unitary in nature and tend to minimize the differences among the various classes of drugs.

It has been proposed (Badiani 2013) that the overall rewarding effects of addictive drugs are determined not only by their ability to produce euphoria ('flash') or to activate the neural mechanism of incentive salience (Berridge et al. 2009) but by a complex interaction between their central and peripheral effects and the setting of drug use. Cocaine, for example, produces, in addition to the characteristic 'flash', a state of arousal by activating noradrenergic transmission both centrally (locus coeruleus) and peripherally (sympathetic nervous system) (Billman 1995; Sofuoglu and Sewell 2009). This state of central and peripheral arousal (characterized by alertness, anxiety, aggressivity, tachycardia, tachypnea, etc.) usually occurs when the individual is exposed to exciting, potentially dangerous contexts. When cocaine is taken at home, the exteroceptive information signalling a safe environment conflicts with the interoceptive information signalling danger. Thus, a *mismatch* between exteroceptive and interoceptive information is produced. The mismatch is much reduced or absent when cocaine is used outside the home environment. According to Badiani's hypothesis, the mismatch reduces the positive valence of using cocaine at home relative to non-home environments.

The reverse line of reasoning applies to heroin. Indeed, except for the powerful 'flash', the effects of heroin are in many respects the opposite of those of cocaine. Heroin depresses the central nervous system (producing sedation and anxiolysis and depressing respiration) and acts in complex manner on the periphery producing, among other effects, bradycardia (Haddad and Lasala 1987; Thornhill et al. 1989). When heroin is taken at outside the home, there is a mismatch between exteroceptive information requiring alertness and vigilance

and interoceptive information signalling reduced arousal and relaxation, whereas there is no mismatch when heroin is used at home. Therefore, the mismatch reduces the positive valence of using heroin outside the home relative to the home environment.

In summary, the setting of drug use provides "an ecological backdrop" against which the central and peripheral effects of drugs are appraised (Badiani 2013). When a "mismatch" between exteroceptive and interoceptive information is detected, the rewarding effect of the drug is thwarted (Badiani 2013). Putting this hypothesis to test in real world addicts presents a series of major challenges. In the next two sections, I will examine the methodological issues concerning the measure of drug effects and emotional valence.

### 1.2. Subjective appraisal of peripheral and central drug effects

As detailed in the previous section, heroin and cocaine produce, in addition to a pleasurable 'flash', a number of central and peripheral effects. Cocaine, for example acts centrally to produce arousal, anxiety, aggressivity, suppression of thirst and appetite, and activates the sympathetic nervous system, thus producing effects such as tachycardia, tachypnea, hypertension, mydriasis, reduced salivation, muscular tension, etc. (Hallman et al. 2012a; Billman 1995; Sofuoglu and Sewell 2009; Antoniazzi et al. 2017). The effects of heroin are very different, as it depresses the central nervous system, thus producing sedation, anxolysis, and respiratory depression, and acts peripherally (partly via the parasympathetic nervous system) to produce bradycardia, hypotension, miosis, and constipation (Larson. 2008, Haddad and Lasala 1987; Thornhill et al. 1989; Nilsson et al. 2016). In summary, while the peripheral effects of cocaine can be

summarized as sympathomimetic, those of heroin are parasympathomimetic-like.

The role of physiological changes in the appraisal of emotion has been hypothesized first by William James (1884) and then developed in different ways by various theorists, most remarkably by Rober Zajonc who hypothesized that emotional appraisal does not require conscious appreciation of the stimuli (1980). Yet, it is often forgotten that the subjective appraisal of the activity of the autonomic nervous system contributes to the self-assessment of one's own state of wellbeing and to emotional processing (Kreibig 2010). Owing to the dichotomy in the involvement of the sympathetic versus the parasympathetic nervous system in emotional processing (Kreibig 2010), it would important to verify the ability of cocaine and heroin co-abusers to appraise the distinctive peripheral effects of the two drugs and to rate their intensity. In particular, we were interested in the subjective appraisal of peripheral effects associated positively or negatively with arousal: increased or decreased heart rate (tachycardia or bradycardia), increased or decreased respiratory rate (tachypnea or bradypnea), increase or decrease in muscular tension. As detailed in the Methods section, the magnitude of these effects was measured using a 5-point Likert scale.

### 1.3. Subjective appraisal of emotional valence

The study of affective states presents a major challenge in neuroscience. This is partly due to the multiplicity of theories and constructs of emotions and to the availability of several alternative measures. However, the general consensus is that there is not a single gold-standard method for measuring affective states (Scherer 2005). Researchers have been using both objective measures of

emotional state: non-verbal behaviour (e.g. facial and vocal expression) and psychophysiological indicators (e.g. skin conductance, heart rate, respiration rate), and subjective measures such as self-report questionnaire. As noted by Scherer (2005) while objective measures can be used to infer the emotional state of a person, there is not an objective method of access to the subjective experience other than to ask an individual to report about the nature of the experience itself.

The limits and the reliability of self-reports are mainly due to the fact that emotions are described using terms of natural language, and this might represent a potential issue in the translation of concepts due to cross-cultural differences and language-specific conceptualizations. Another critical point is related to the existence of unconscious affective reactions of which the person might be simply not aware, even when explicitly asked to report on their conscious mental state (Winkielman and Berridge 2003). Furthermore, asking the respondent directly to focus the attention on his/her own experience might induce a retrospective judgment of the experience itself, which can distort the declared emotion (Köster and Mojet 2015). This last point can become especially relevant in the field of drug addiction, where the social undesirability and stigma related to drug use may lead the respondents to conceal certain behaviours. However, evidence suggests that self-reports offer a sufficiently reliable, valid and legitimate method for studying the behaviour of illicit drug users (for a review see Darke 1998).

Affective neuroscientists in order to obtain self-report of emotional experience usually adopt one the following approaches: 1) the discrete emotional approach, and 2) the dimensional approach.

The discrete approach is based on the idea, first proposed by Darwin (1872), that basic emotions reflect universal psychobiological responses, which can be treated as separate and independent entities. Conventionally, it relies on the semantic categorization present for each emotion in the natural language. Usually, the self-report is composed by a checklist of emotional terms, and the individual is asked to rate whether the respective emotion was experienced, on a nominal, ordinal, or interval scale (Ekman, 1999; Izard, 1992).

The dimensional approach was first proposed by Wundt (1874) in an attempt to use introspection in the experimental setting. Wundt introduced the idea that internal subjective feelings can be described by their position on continuous multidimensional space. Modern emotional theorists have developed different dimensional models (Larsen & Diener, 1992; Russell, 2003; Schlosberg, 1952; Watson et al. 1999) and although they are still poorly represented in neuroscience and psychiatry, they could offer valuable insight in pathologies that involve a disorder of affects (Posner et al. 2005).

Clinicians and researchers are aware of the difficulty that some people experience in assessing, discerning, and describing their emotional states (Saarni 1999). This may be in part due to fact that affective states are often highly correlated and lack defined borders (Russell and Fehr 1994). Furthermore, the subjective experience of neurophysiological changes of pleasure and arousal tend to be labeled as a cognitive interpretation in relation to an eliciting stimulus, and within situational contexts, culminating in the subjective experience of a specific affective state (Russell 2003). To overcome these limitations, in the present study we adopted the Circumplex Model of Affect proposed by Russell (1980) as a theoretical framework to investigate the relation between drug and setting.

The Circumplex Model of Affect posits that all affective states arise from core sensations produced by two independent neurophysiological systems: arousal (along high-low energy continuum) and valence (pleasure-displeasure continuum). From this perspective, each emotion arises as a linear combination of these two dimensions, or as varying degrees of pattern of activation of both valence and arousal. The Circumplex model has shown reliability and validity across cultures (Russell 1983). Using standardized objective probes, researchers have observed that peripheral physiological responses to affective stimuli vary incrementally accordingly with subjective ratings of valence and arousal. For instance, it has been shown a high correlation in the increase of skin conductance and heart rate accelerations with subjective ratings of arousal (Lang et al. 1993). Similarly, subjective valence ratings have been correlated with facial electromyographic (EMG) measurements of changes in musculature contraction (Cacioppo et al. 1986; Lang et al. 1993).

**1.4. Aims of the present study**

The present study had two major aims. In Experiment 1, we verified the ability of heroin and cocaine co-abusers to appraise subjectively the distinct spectra of peripheral effects produced by the two drugs. In Experiment 2, we verified the hypothesis that the affective states produced by cocaine and heroin undergo a shift in valence as a function of the setting. In particular, it was predicted that the affective valence of heroin would be more positive when the drug is taken at home than when is taken outside the home. The opposite should occur for cocaine.

## 2. Materials and Methods

### 2.1. Participants

All procedures and methods were approved by the *University of Sussex Science and Technology Cross-Schools Research Ethics Committee (C-REC).* Participation in the studies was voluntary, and no monetary or non-monetary incentives were offered. Prospective participants were recruited during their daily visit at the Substance Misuse Services of Villa Maraini (Rome, Italy) by the social workers and the medical staff and invited to participate in the study. Prospective participants were then screened to exclude major psychiatric disorders and severe alcohol dependence (as indicated by a state of inebriation at recruitment or by treatment for alcohol abuse). Inclusion criteria included: age between 18 and 68 years; at least 12 consecutive months of heroin and/or cocaine use; fixed residence at time of regular drug use; good understanding of the Italian or English language. After the study had been described to the prospective participants, informed consent was obtained before the start of the study. Participants were informed that the questionnaires focused on questions about their current (or past) experience with heroin and cocaine. Furthermore, the participants were assured that data were anonymous and confidential, that they were free to withdraw from the study at any time they wished, and that they were allowed to skip all questions they were not comfortable to answer. The questions were presented in a fixed order between participants with the question relative to heroin before cocaine. This method was chosen based on previous pilot interviews in order to avoid carryover effects of the recollection of cocaine experiences.

*2.1.1. Experiment 1*

Fifty-one addicts (mean age = 35.96, *SD*=9.99), who self-identified as females (12) or males (39), participated in this experiment. All participants had a long history of heroin (15.12 years, *SD*=9.36) and cocaine (14.15 years, *SD*=7.88) co-abuse. Nearly all participants (97.96%) had made heavy use of street heroin in their life (i.e., daily use for 3 or more months). Most of them (97.83%) were enrolled in methadone programs (59.66 mg/day, *SD*=53.60) but current heroin use (i.e., at least one episode in the last three months) was still prevalent (78.43% of subjects). The main route for heroin administration was intravenous injection (46.81%), followed by insufflation (i.e., intranasal administration; 27.66%) and inhalation (smoking-'chasing the dragon' method; 25.53%).

Most participants (77.55%) had made also heavy use of cocaine and 70.83% of them were still current users (i.e., at least one episode in the last 3 months). The main route for cocaine administration was insufflation (37.23%), followed by inhalation (smoking-'water bottle' method; 35.11%) and intravenous injection (27.66%).

Table 1 summarizes the socio-demographic characteristics of the sample and basic information about drug use.

*2.1.2. Experiment 2*

Fifty-three addicts (mean age = 37.11, *SD*=10.42), who self-identified as females (11) or males (42), participated in this experiment (43 of them also participated in Experiment 1). The sample included 45 heroin and cocaine co-abusers, 7 were cocaine-only abusers and 1 heroin-only abuser. They all had a long history of heroin (15.96, *SD*=10.31) and/or cocaine (14.44, *SD*=8.51) use. Nearly all participants (97.83%) had been heavy users of street heroin. Most of them

(84.91%) were enrolled in methadone programs (59.66 mg/day, *SD*=53.60) but current heroin use was still prevalent (73.91% of subjects). Also in this group, the main route for heroin administration was intravenous injection (54.35%), followed by inhalation (23.91%) and insufflation (21.74%).

Most participants (73.08%) reported heavy use of cocaine at some time in life and 55.77% of them were current users. The main route for cocaine administration was insufflation (48.00%), followed by inhalation (26.00%), and intravenous injection (26.00%).

Table 1 summarizes the socio-demographic characteristics of the sample and basic information about drug use. The majority of the participants (86.79%) had a fixed residence at the time of their enrolment in the study. However, it is important to point out that the information concerning the setting of drug use referred to periods in which the participants had a fixed residence.

**2.2. Data collection**

The data were collected using the online survey host Survey-Gizmo in a quiet testing room. Computerized interview methodology is considered a faster, more relaxing, and more engaging way to collect data from alcohol and drug users relative to other forms of self-report (Skinner et al. 1983). The data were entered using the offline mode by the interviewer. In addition to the general information described in the previous section, we collected the data detailed in the next sections.

*2.2.1. Experiment 1*

Participants were informed that the survey focused on questions about their current (or past) experience with heroin and cocaine. The general instruction was to recall a *typical* drug experience and to rate the magnitude of the

perceived effect. Participants were instructed to exclude instances of combined heroin and cocaine use ('speedball').

> *"In this part of the survey, you will be asked to rate the physiological changes of which you are aware of when under the effect of a specific drug. Please rate to what extent each change applies to your typical experience with* ***heroin*** *and/or* ***cocaine****.*
> *The alterations can be in both directions (****Increase – Decrease****)."*

In addition, to changes in heart rate, respiratory, and muscular tension we also assessed the subjective appraisal of intestinal function (bowel movements), urinary function, visual acuity, salivation, sexual drive, and appetite.

The magnitude of the perceived effect (relative to baseline conditions, that is, in an undrugged state) was rated using a bi-directional 5-point Likert scale, with the anchors 1=small-effect, 5 = large-effect (0 = no perceived effect).

*2.2.2. Experiment 2*

The participants were instructed to recall a typical drug experience and to rate the affective state produced by heroin versus cocaine in two setting (at home versus outside the home). Also in this case, the participants were instructed to exclude instances of combined heroin and cocaine use ('speedball').

The emotional state induced by the drug was assessed using a graphic approach based on the Circumplex Model of Affect (Russell 1980; see Figure 1, left panel), already described in Section 1. Our aim was to develop a user-friendly, intuitive test that could be completed rapidly without relying on the cognitive processes required for the verbal description and interpretation of emotional states (see Schrerer et al. 2001), which could have represented a confounding factor given the addicts' negative feelings about their own addiction (Dearing et al. 2005; Luoma et al. 2012, Luoma et al. 2013). Thus, we

created the diagram illustrated in the right panel of Figure 1, representing a two-dimensional space of emotional state with arousal on the vertical dimension and valence on the horizontal dimension. Emoticons and colours were added to increase the evocative power of the diagram (Kaye et al. 2016; Nathanson et al. 2016).

For each combination of drug and setting the participant was asked to choose the quadrant that best reflected the affective states experienced while under the influence of the drug: i) top-right yellow quadrant if the emotional state was simultaneously pleasurable and arousing; ii) bottom-right green quadrant if the emotional state was simultaneously pleasurable and sedating; iii) bottom-left blue quadrant if the emotional state was simultaneously unpleasant and sedating; (iv) top-left red quadrant if the emotional state was simultaneously unpleasant and arousing.

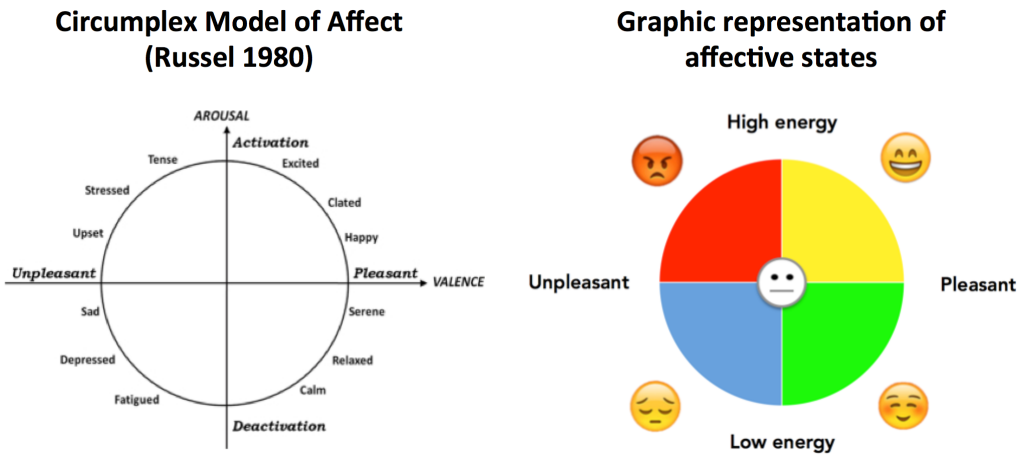



**Figure 1. Emotional Model of Arousal and Valence. Left Panel:** Graphic representation of the Circumplex Model of Affect (Russel 1980). **Right panel:** Bidimensional representation of affective states used in Study 2. This test was developed based on the Circumplex Model of Affect illustrated in the left panel, by removing the labels indicating different levels for each dimension, and by adding emoticons.

We expected that in some case the affective state experienced while under the effect of the drug could not be the reduced to a single condition. For

this reason and in order to not affect the ecological validity of the data, no restriction was placed on the number of affective states that the participant could report for each combination of set and setting.

## 2.3. Data analysis and Statistics

### *2.3.1. Experiment 1*

The scores for each effect were compared with a pre-specified null hypothesis (0 change) using one-sample Mann-Whitney test. The two-tailed Wilcoxon signed rank test for paired data was used to assess differences between heroin and cocaine scores for each effect. We also calculated the Pearson's correlation as a measure of effect size. According to Cohen (1992), a value of 0.1 is considered a small effect, 0.3 a medium effect and a value of 0.5 or greater, a large effect size. All analyses were performed using R (version 3.3.3) Statistical Software (R Core Team 2014).

### *2.3.2. Experiment 2*

In this experiment, the participants were allowed to select, for each combination of drug and setting, one or more of the four quadrants of the diagram illustrated in Figure 1. As shown in Table 3, in 82.7% of cases the participants indicated a single quadrant, more rarely two quadrants (15.3%) and only in 2% of cases they selected three or four quadrants. In 11.2% of cases the selection of more than one quadrant resulted in a mixed valence. That is, in 88.8% of cases the valence was either entirely pleasant or entirely unpleasant. These data were classified and analysed in three different ways in order to test three separate hypotheses.

*2.3.2.1. The 'pleasure' hypothesis.* As discussed in Chapter 1 and in Section 1, it is often assumed that all drugs are the same in producing 'pleasure'. For each combination of drug and setting, we calculated the observed frequency of the following three categories: i) pleasant (by combining the frequency of entries for quadrants 'pleasant-arousing' and 'pleasant-sedating'), ii) unpleasant (by combining the frequency of entries for quadrants 'unpleasant-arousing' and 'unpleasant-sedating'), and iii) mixed valence (for all the instances in which both pleasant and unpleasant quadrants were selected). We used the one-sample Kolmogorov-Smirnov test (Massey 1951) to assess the degree to which the observed frequencies differed from the expected frequencies based on the null hypothesis (pleasant:mixed:unpleasant = 1:0:0). We also tested a 'weak' version of the 'pleasure' hypothesis in which the categories pleasant and mixed were combined (pleasant/mixed:unpleasant = 1:0).

*2.3.2.2. Shift in valence (hypothesis 1).* The working hypothesis (see Section 1.1) predicted a complete or partial shift in the affective valence of heroin and cocaine as a function of setting. The data were arranged in a 2x2 contingency table and the McNemar's test was used to assess the difference between the two correlated proportions (McNemar 1947). Of course, only co-abusers were included in this analysis.

*2.3.2.3. Shift in valence (hypothesis 2).* A more rigorous reading of the working hypothesis would require limiting the analysis to the individuals who experienced heroin-induced sedation in both settings and to those who experienced cocaine-induced activation in both settings. That is, all cases in

which there was a discrepancy for the dimension 'arousal' were excluded from the analysis. Also this hypothesis was tested using the McNemar's test.

## 3. Results

### 3.1. Experiment 1

As illustrated in Table 2 and Figures 2 and 3, the profile of the subjective effects of heroin and cocaine were very different, consistent with the working hypothesis. It should be noticed that the perceived drug effects of heroin and cocaine were rarely unidirectional, that is, for most measures some participants reported an increase and others a decrease (see Table 2 and Figure 2). The only exception concerned cocaine, which increased heart rate, respiratory rate, and muscular tension in most participants, whereas no participant reported the opposite.

#### *3.1.1. Heart rate: opposite changes induced by cocaine vs. heroin*

Nearly all participants (98%) reported an increase in heart rate when under the influence of cocaine; not surprisingly the ratings were significantly different from zero ($p<0.001$). In contrast, heroin tended to decrease heart rate ($p<0.01$), even though only 33% subject reported this effect whereas 63% reported no change and 4% an increase. There was a significant difference between the scores for cocaine and those for heroin ($Z=5.86$, $p<0.0001$, $r=0.8$).

*3.1.2. Respiratory rate: opposite changes induced by cocaine vs. heroin*

Cocaine increased their respiratory rate ($p<0.001$), as reported by 80% of participants, whereas heroin tended to reduce it ($p<0.01$), even though only 41% of participants reported this effect. Also in this case, there was a significant difference between the scores for cocaine and those for heroin ($Z=-6.15$, $p<0.0001$, $r=0.9$).

*3.1.3. Muscular tension: opposite changes induced by cocaine vs. heroin*

The majority of participants reported opposite changes in muscular tension for heroin vs. cocaine. Cocaine increased muscular tension ($p<0.001$) in 78% of participants whereas heroin decreased it ($p<0.001$) in 82% of participants. Again, there was a significant difference between the scores for cocaine and those for heroin ($Z=5.78$, $p<0.0001$, $r=0.8$).

*3.1.4. Intestinal function: opposite changes induced by cocaine and heroin*

As expected, the majority of subjects (74%) reported that heroin decreased their intestinal function ($p<0.001$), a well-known effect of opioid agonists (Camilleri 2011; Holzer 2009). Surprisingly, 45% of the participants reported an increase in intestinal function after cocaine ($p<0.05$), probably due to adulterants present in street-grade cocaine, such as mannitol (Cunningham et al. 1984). There was a significant difference between the scores for cocaine and those for heroin ($Z=5$, $p<0.0001$, $r=0.7$).

*3.1.5. Urinary function: modest reduction by heroin but not cocaine*

There was no significant change in urinary function after cocaine ($p$=0.31) and modest decrease after heroin ($p$<0.05), consistent with previous reports (Redan et al. 2016). However, there was a significant difference between the scores for cocaine and those for heroin ($Z$=3.14, $p$=0.002, $r$=0.4).

*3.1.6. Salivation: decreased by both cocaine and heroin*

Salivation was decreased by cocaine ($p$<0.001) and, to a lesser degree, by heroin, with significant differences between the scores for the two drugs ($Z$=2.06, $p$=0.03, $r$=0.3).

*3.1.7. Appetite: suppressed by cocaine*

Not surprisingly, the majority of the participants (87%) reported decreased appetite after cocaine (Cochrane et al. 1998; Ersche et al. 2013) whereas there was no significant change after heroin ($p$=0.73), with 34% of participants reporting a decrease, 30% an increase, and the 37% no change in appetite. There was a significant difference between the scores for cocaine and those for heroin ($Z$=4.34, $p$=0.0001, $r$=0.6).

*3.1.8. Visual acuity: reduced by heroin but not by cocaine*

The participants reported a modest but highly significant decrease in visual acuity after heroin ($p$<0.001) but no change after cocaine, heroin, with no significant differences between the scores for the two drugs ($Z$=1.65, $p$=0.09 ns, $r$=0.2).

*3.1.9. Sexual drive: no changes*

There was no significant change in sexual drive after either cocaine or heroin use. This was somewhat surprising, given that cocaine is thought to increase sexual desire (Johnson et al. 2017).

## 3.2. Experiment 2

*3.2.1. Drug 'pleasure'*

As illustrated in Table 3 and Figure 4, the overall valence of the typical drug experience was not always pleasurable, with important differences as a function of both drug and setting. When heroin was taken at home, the majority of participants (89.1%) experienced a pleasant affective state, whereas only 6.5% reported an unpleasant state and 4.3% a mixed state (both pleasant and unpleasant). The observed frequencies were not significantly different ($p$>0.2) from those expected on the basis of either the strong (pleasant:mixed:unpleasant = 1:0:0) or the weak version (pleasant/mixed:unpleasant = 1:0) of the 'pleasure' hypothesis (Table 5). In contrast, when heroin was used outside the home the overall experience was pleasant in only 39.1% of participants, whereas 50% reported an unpleasant experience. These frequencies differed significantly from both the strong and the weak version of the 'pleasure' hypothesis ($p$<0.0001).

Cocaine use at home produced a pleasurable state only in 26.9% of participants whereas 61.5% experienced an unpleasant state and 11.6% a mixed state. These frequencies differed significantly from both the strong and the weak version of the 'pleasure' hypothesis ($p$<0.0001). Also when taken outside the home cocaine resulted in an unpleasant experience for a sizeable number of participants (32.7%) and only 50% reported a pleasant state from both the

strong and the weak version of the 'pleasure' hypothesis ($p$<0.0001). Also in this case, the observed frequencies differed significantly from both the strong and the weak version of the 'pleasure' hypothesis ($p$<0.0001).

In summary, only in the case of heroin use at home, the emotional state induced by the drug was rated as overall pleasant by the majority of participants. This is not consistent with the notion that all addictive drugs produce a pleasurable affective state.

*3.2.2. Shift in valence (hypothesis 1)*

As illustrated in Table 5, in 56.5% of participants the valence shifted from mainly positive at home to mainly negative outside the home, in agreement with the main prediction of our working hypothesis. Only in 2.2% of participants (1 individual) the shift was opposite to the predicted one. The McNemar's test indicated that the shift was highly significant ($p$<0.0001). In 41.3% of participants there was no shift in valence.

Also in the case of cocaine (see Table 5), the McNemar's test indicated a significant shift in valence as a function of setting ($p$=0.0014). For 48% of participants, the valence shifted from mainly negative at home to mainly positive outside the home, in agreement with the main prediction of our working hypothesis. Only in 12% of participants, the shift was opposite to the predicted one. In 40% of participants there was no change in valence as a function of setting.

*3.2.3. Shift in valence (hypothesis 2)*

The working hypothesis predicted that the shift in valence was the result of a mismatch between exteroceptive and interoceptive information (a mismatch

arousing effects of cocaine in a home environment and the sedative effects of heroin outside the home). The data were thus re-analyzed, including only the case in which there was concordance for the vertical dimension (see Figure 1). Also this sub-set of data was consistent with the working hypothesis, as the McNemar's test indicated that a significant shift in valence as a function of setting for both heroin ($p<0.001$) and cocaine ($p=0.0015$).

## 4. Discussion

The present study investigated the subjective effects of heroin and cocaine in drug addicts who co-abused both drugs. I report here three major findings. First, we found that heroin and cocaine produced very different spectra of subjectively perceived central and peripheral effects. Second, we found that the affective state produced by heroin and cocaine is not always pleasurable. Third, we found that the affective state produced by heroin and cocaine can undergo a shift in valence as a function of setting.

### 4.1. Subjective appraisal of central and peripheral effects of heroin and cocaine

In Experiment 1 we assessed the subjective appraisal of central and peripheral effects of heroin and cocaine, as reported by experienced co-abusers. The direction and magnitude of these changes are illustrated in Table 2 and Figures 2 and 3. To the best of my knowledge, no previous study has compared the subjective effects of cocaine versus heroin in human addicts.

The spectra of effects of the two drugs were very different and in many cases virtually the opposite. The majority of participants reported that under the influence of cocaine they perceived an increase in heart rate, respiratory rate, and muscular tension, as well as a decrease in salivation. These subjective effects are consistent with previous reports from the literature (Hallman & Lyskov 2012, Billman 1995, Sofuoglu and Sewell 2009, Maceira et al. 2014; Antoniazzi et al. 2017) and can be easily attributed to the blockade of the norepinephrine transporter on the terminals of the sympathetic nervous system. In contrast the same co-abusers reported that under the influence of heroin they perceived a reduction in heart rate and respiratory rate. The mechanisms responsible for these effects are only partly known and might involve both central (depression of the bulbar respiratory centres) and peripheral mechanisms (including parasympathetic and sympathetic mechanisms) (Haddad and Lasala 1987; Thornhill et al. 1989). The participants also reported a reduction in muscular tone, which may be related to the depression of the central nervous system and/or to the anxiolytic effect and/or to the analgesic effect produced by heroin. However, to best of my knowledge, there are no scholarly reports that can shed light on the mechanisms responsible for this subjective effect. In summary, regardless of the exact mechanisms involved, cocaine produced classical sympathomimetic effects whereas heroin produced parasympathomimetic-like effects. The importance of this dichotomic pattern lies in the role played by the autonomic nervous system in regulating emotional processing (Levenson 2014; Kreibeg 2010), which will be discussed in Section 4.3 below.

There were also significant differences for other subjective effects of cocaine and heroin: intestinal function, urinary function, salivation, and

appetite. While these differences might have contributed to create distinct spectra of subjective effects in response to heroin versus cocaine, it is unlikely they might have contributed to determine the substance-specific interaction between drug and setting.

### 4.2. Drug 'pleasure'

Although it was not the major aim of the present study, the collection of data concerning the emotional valence of the drug experience gave us the opportunity to address a contentious issue in the field of drug addiction research. As discussed in Chapter 1, it is often thought even by experts (Wise 1980, 2008) that all addictive drugs "directly or indirectly target the brain's reward system by flooding the circuit with dopamine […] in regions of the brain that regulate […] feelings of pleasure. The overstimulation of this system, which rewards our natural behaviors, produces the euphoric effects sought by people who use drugs and teaches them to repeat the behavior." (https://www.drugabuse.gov/publications/media-guide/science-drug-abuse-addiction-basics). However, two decades of animal and human studies have shown that the hedonia/anhedonia dopamine hypothesis is incompatible with experimental findings and that addictive drugs can affect in a very different manner distinct aspects of the rewarding process as suggested by Berridge and colleagues (Smith et al. 2011 Berridge et al. 2009; Berridge 2012; Berridge and Kringelbach 2013).

Our data not only indicate that even prototypical addictive drugs like heroin and cocaine do not produce necessarily a pleasurable affective state in all contexts. Actually, we have shown that in certain settings almost two-thirds of experienced drug users report that cocaine produces a mainly unpleasant

affective state even during the period of regular use. This is consistent with reports by Ettenberg and colleagues (2004, 2009) that heroin elicits pure approach behavior in the rat, whereas cocaine elicits more complex approach-avoidance behaviour.

### 4.3. Shift in affective valence as a function of setting

Studies in rats, already reviewed in Chapter 1 and in the previous sections of this Chapter, have shown that drug preferences are a function of setting, heroin being rewarding at home than outside the home and cocaine being more rewarding outside the home than at home (Caprioli et al. 2007, 2008). Also in humans, drugs and settings are associated in a substance-specific manner. Heroin and cocaine co-abusers tend in fact to prefer (albeit not exclusively) the home environment for heroin use and non-home environments for cocaine use (Caprioli et al. 2009; Badiani and Spagnolo 2013). On the basis of these and other findings, it has been proposed the rewarding effects of addictive drugs are the results of complex interaction among central and peripheral effects and the setting of drug taking (Badiani 2013). In particular, it has been proposed that the affective valence of cocaine is thwarted when the drug is taken in a home environment because of the mismatch between exteroceptive information (i.e., safe home environment) and interoceptive information (i.e., the central and peripheral arousal produced by cocaine). Similarly, the affective valence of heroin is thwarted when the drug is taken in an exciting, potentially dangerous environment because of the mismatch between the latter and the state of sedation produced by heroin. Preliminary support for this hypothesis was provided by a study in rats in which it was found, using 50 kHz ultrasonic vocalizations as an index of positive affect, that the affective valence of cocaine

and heroin reward was modulated by the setting (Avvisati et al. 2016): rats vocalized more for heroin than for cocaine at home, whereas they vocalized more for cocaine then heroin outside the home. The main goal of the present study was to investigate if the same type of environmental modulation occurs in human addicts. The findings reported here indicate that this is indeed the case.

Using a novel bi-dimensional test developed on the basis of the Circumplex Model of Affect (Russel 1983), we found that the affective state produced by heroin was indeed appraised as more pleasant when the drug was used at home then when used outside the home, whereas the affective state produced by cocaine was more pleasant when the drug was used outside the home then when used at home. More specifically, our data confirmed that the shift in the affective valence of heroin occurred in association with its sedative effects (that is, a shift was observed in the subset of individual reporting sedation after heroin), whereas the shift in the affective valence of cocaine occurred in association with its arousing effects (that is, a shift was observed in the subset of individual reporting arousal after cocaine). It is reasonable to assume that the sympathomimetic effects of cocaine and the parasympathomimetic-like effect of heroin (see Section 4.1) contributed to generate the emotional state of arousal and sedation produced by cocaine and heroin, respectively.

Taken together these results are in agreement with the hypothesis that a mismatch between interoceptive and exteroceptive information decreases the positive valence of drug experience (Badiani 2013). As pointed out by Badiani (2013) "It is important to emphasize that emotional appraisal does not necessarily entail the conscious elaboration of stimuli (Zajonc 1980, Chritchley

2009, Gray et al. 2012) which, incidentally, would be difficult to envisage in rats".

### 4.4. Conclusions

Further studies are necessary to investigate the causal relationships in the interaction between exteroceptive and interoceptive information relevant to the appraisal of drug reward. However, the results reported here strongly suggest a major role of interoception in modulating the affective response to addictive drugs.

In the last few years, there has been a growing interest in the role of interoception in emotional processing. The term interoception, first introduced by Sherrington (1906), refers to the "sense of the physiological condition of the body", and is thought to be "influenced by the dynamic state of physiological arousal" (Craig 2002; Herbert et al. 2012). Interoception is crucially important for homeostasis, but does not necessarily involve the cognitive appraisal of the information. In contrast, interoceptive awareness, which requires direct conscious attention to internal bodily sensations, is thought to be important for cognitive processes involved in emotional self-regulation (Mehling et al. 2009). In particular, recent clinical research in drug addiction has suggested an important role of interoception for emotional self-regulation (Goldstein et al. 2009; Paulus et al. 2009, Noël et al. 2013; Paulus and Stewart 2014; Price and Smith-DiJulio 2016).

In Chapter 4, I will discuss the implications of the present findings for the development of novel therapeutic approaches to the treatment of substance use disorders.

Table 1. Socio-demographic information

| | Study 1 (N= 51) *mean (SD)* | Study 2 (N= 53) *mean (SD)* |
|---|---|---|
| **Drug use** | | |
| • Heroin and cocaine | 100% | 84.91% |
| • Heroin only | 0% | 1.89% |
| • Cocaine only | 0% | 13.21% |
| **Age (years)** | 35.96 (9.99) | 37.11 (10.42) |
| **Sex/gender (females)** | 24% | 21% |
| **Education (years)** | 11.68 (3.67) §a | 11.21 (3.47) §b |
| **Ethnicity** | | |
| • Caucasian | 86.27% | 88.68% |
| • Multiethnic | 9.80% | 7.55% |
| • Black/African | 1.96% | 1.89% |
| • Indian/Pakistani/Bangladeshi | 1.96% | 1.89% |
| **Employment status⌘** | | |
| • Employed | 48.71% | 46.34% |
| • Retired/Disability | 5.13% | 9.76% |
| • Controlled environment | 5.13% | 9.76% |
| • Unemployed | 41.03% | 34.15% |
| **Fixed Residence** | 86.27%◆a | 86.79% |
| **Household** | | |
| • Family/partner | -- | 82.61% |
| • Alone | -- | 10.87% |
| • Flatmates | -- | 6.52% |
| **City of residence (no. inhabitants)** | | |
| • <100.000 | 89.80% | 90.57% |
| • 10.000-100.000 | 10.20% | 9.43% |
| **Methadone program** | 97.83% | 84.91% |
| mg/day | 59.66 (53.60) #a | 55.91 (57.24) #b |
| **Heroin** | °a | |
| Years of use | 15.12 (9.36) | 15.96 (10.31) |
| Main route of administration | | |
| • Intravenous injection | 46.81% | 54.35% |
| • inhalation (smoked) | 25.53% | 23.91% |
| • insufflation (snorted) | 27.66% | 21.74% |
| **Cocaine** | | |
| Years of use | 14.15 (7.88) | 14.44 (8.51) |
| Main route of administration | °a | °b |
| • Intravenous injection | 27.66% | 26.00% |
| • inhalation (smoked) | 35.11% | 26.00% |
| • insufflation (snorted) | 37.23% | 48.00% |

Notes: *Education:* §a Missing data for 14 participants, §b Missing data for 12 participants; *Employment:* ⌘ Missing data for 12 participants; *Fixed Residence:* ◆a Missing data for 2 participants; *Methadone program (mg/day)*: #a Missing data for 15 participants, #b Missing data for 11 participants; *Main routes of administration*: °a Missing data for 2 participants, °b Missing data for 2 participants.

Table 2. Subjective appraisal of the central and peripheral effects of cocaine and heroin.

| | **Increase (% participants)** | | | | | | **Decrease (% participants)** | | | | | | No Effect (% participants) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Cocaine** | | | **Heroin** | | | **Cocaine** | | | **Heroin** | | | **Cocaine** | **Heroin** |
| **Effect** | Large | Moderate | total | Large | Moderate | total | Large | Moderate | total | Large | Moderate | total | | |
| Heart rate*** | 73 | 25 | **98** | 0 | 4 | **4** | 0 | 0 | **0** | 8 | 25 | **33** | **2** | **63** |
| Respiratory rate*** | 39 | 41 | **80** | 2 | 6 | **8** | 0 | 0 | **0** | 14 | 27 | **41** | **20** | **51** |
| Muscular tension*** | 45 | 33 | **78** | 2 | 2 | **4** | 0 | 0 | **0** | 41 | 41 | **82** | **22** | **14** |
| Intestinal function*** | 20 | 25 | **45** | 0 | 2 | **2** | 6 | 14 | **20** | 39 | 35 | **74** | **35** | **24** |
| Urinary function** | 10 | 20 | **30** | 4 | 14 | **18** | 10 | 8 | **18** | 27 | 16 | **43** | **53** | **39** |
| Visual acuity | 18 | 8 | **26** | 4 | 4 | **8** | 16 | 22 | **38** | 6 | 41 | **47** | **37** | **45** |
| Salivation* | 8 | 8 | **16** | 2 | 6 | **8** | 39 | 35 | **74** | 14 | 27 | **41** | **10** | **51** |
| Sexual drive | 12 | 18 | **30** | 12 | 24 | **36** | 12 | 18 | **30** | 14 | 20 | **34** | **43** | **31** |
| Appetite*** | 2 | 6 | **8** | 8 | 22 | **30** | 69 | 18 | **87** | 18 | 16 | **34** | **6** | **37** |

Asterisks indicate significance differences (Wilcoxon signed rank test) between heroin and cocaine; *p<0.05, **p<0.005, ***p<0.0001

**Figure 2.** Percent distribution of individual responses for the subjective appraisal of central and peripheral effects of cocaine and heroin (same data of Table 2).

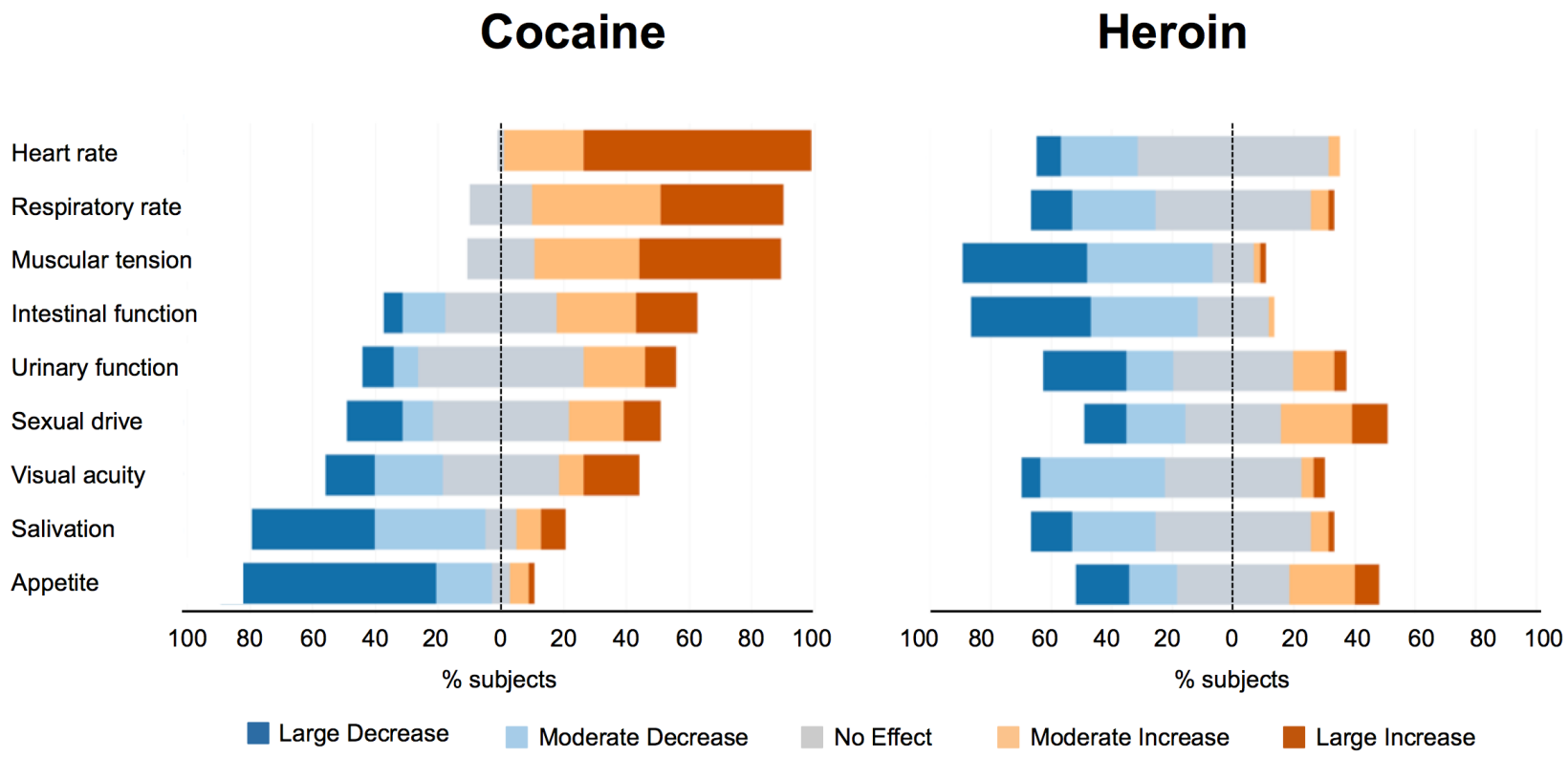


**Figure 3.** Mean score (±SEM) for the appraisal of the effects of cocaine and heroin on the Autonomic Nervous System function and on other systems (same data of Table 2 and Figure 2). Hashtags indicate significant changes from baseline (one-sample Mann-Whitney test); #p<0.05, ##p<0.01, ###p<0.001. Asterisks indicate significance differences (Wilcoxon signed rank test) between heroin and cocaine; *p<0.05, **p<0.01, ***p<0.001

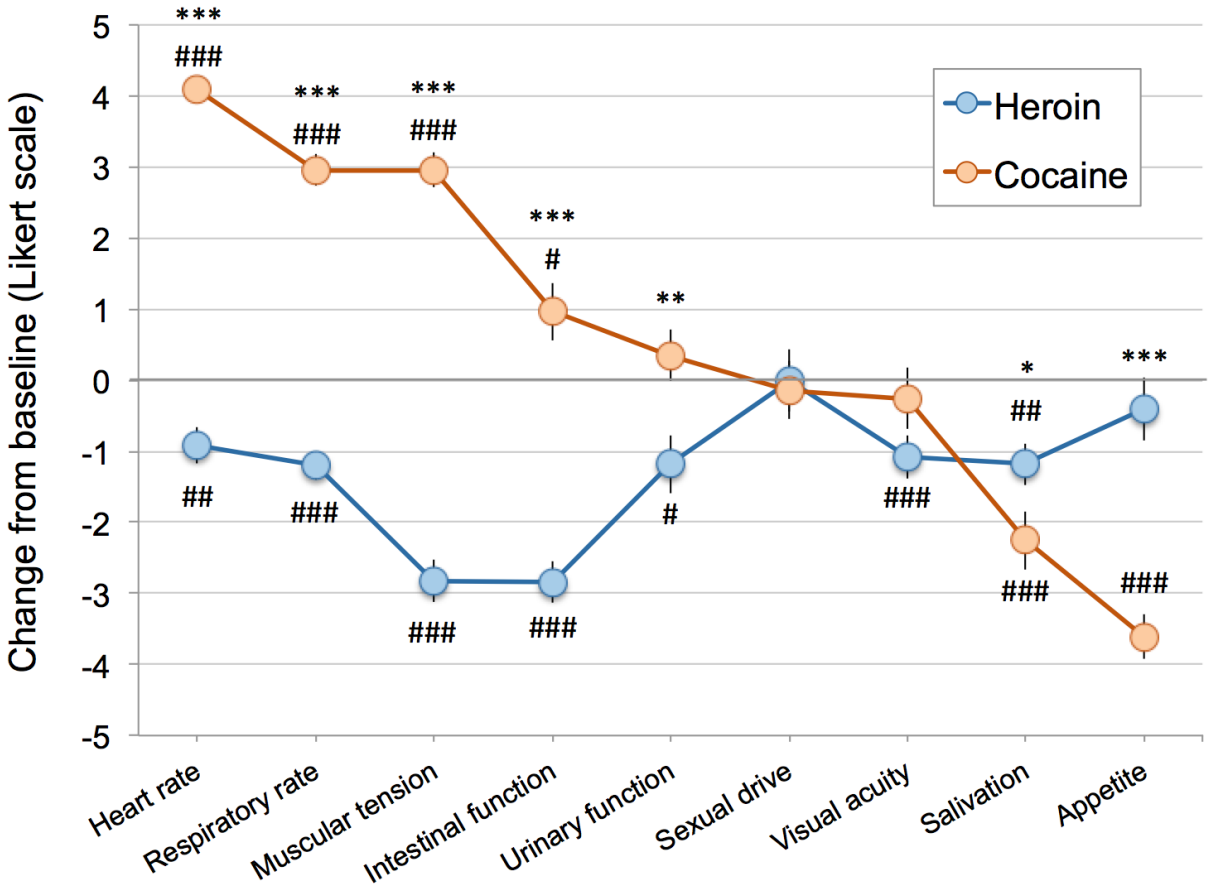


Table 3. Subjective appraisal of the emotional valence of drug experience as a function of drug and setting (same data of Figure 4)

| | Pleasant | | | P/U | Unpleasant | | | P/U | Mix |
|---|---|---|---|---|---|---|---|---|---|
| | High energy | High/low energy | Low energy | Low energy | Low energy | High/low energy | High energy | High energy | |
| Heroin at home | 12 | 4 | 25 | 0 | 2 | 0 | 1 | 1 | 1 |
| | 41 | | | 0 | 3 | | | 1 | 1 |
| Heroin outside the home | 5 | 6 | 7 | 0 | 15 | 1 | 7 | 5 | 0 |
| | 18 | | | 0 | 23 | | | 5 | 0 |
| Cocaine at home | 12 | 0 | 2 | 1 | 8 | 0 | 24 | 3 | 2 |
| | 14 | | | 1 | 32 | | | 3 | 2 |
| Cocaine outside the home | 23 | 1 | 2 | 0 | 1 | 0 | 16 | 8 | 1 |
| | 26 | | | 0 | 17 | | | 8 | 1 |

Figure 4. Subjective appraisal of the emotional valence of drug experience as a function of drug and setting.

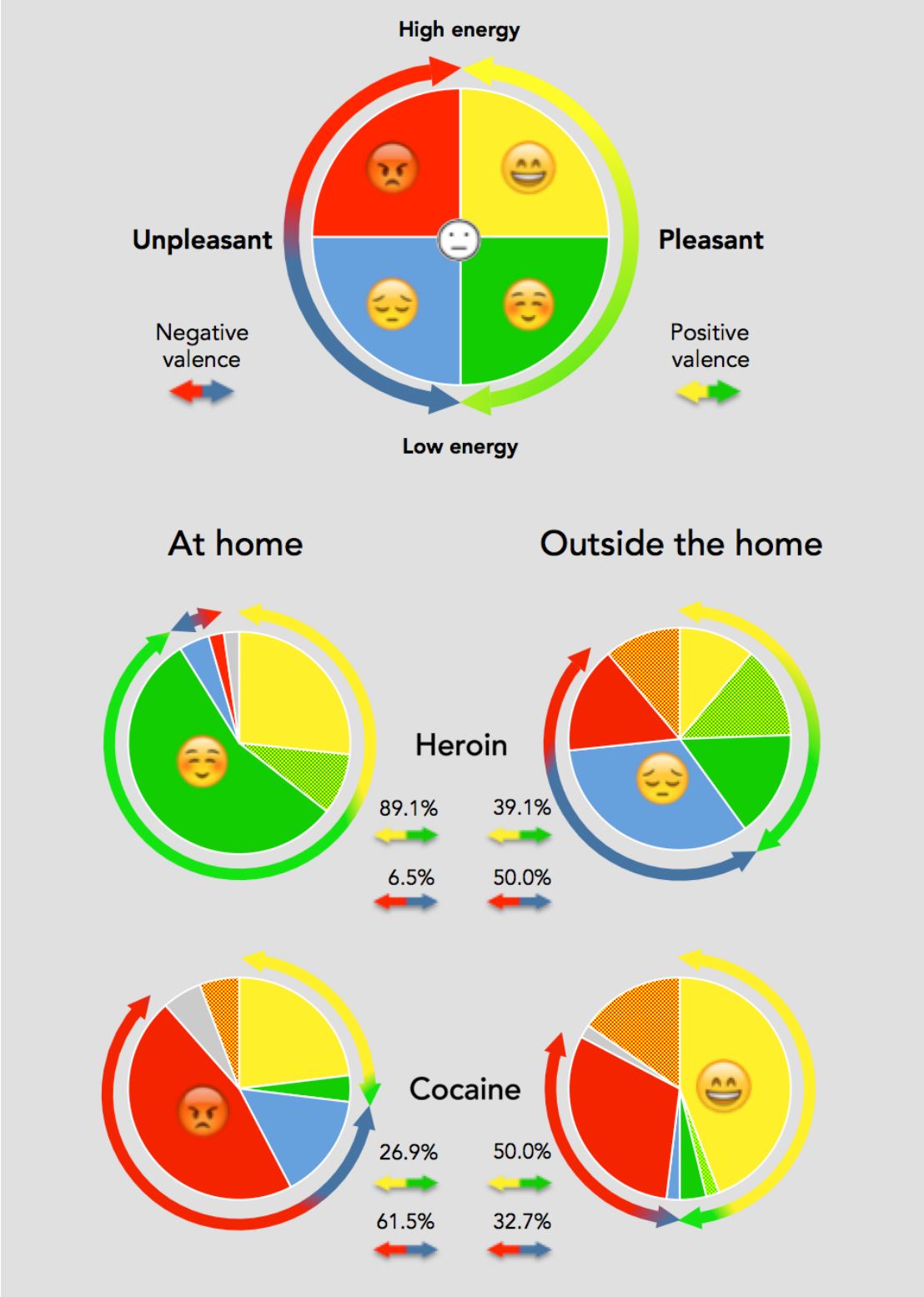


Table 4. Observed subjective appraisal for the emotional valence of drug experience versus expected frequencies (based on the 'pleasure' theory of drug reward), as a function of drug and setting.

| | **Observed frequencies** | | | Kolmogorov-Smirnov one-sample test for the comparison with expected frequencies based on 'pleasure' theory | |
|---|---|---|---|---|---|
| | **Pleasant** | **P/U** | **Unpleasant** | Expected frequencies 'strong' version Pleasant:mixed:unpleasant (1:0:0) | Expected frequencies 'strong' version Pleasant/mixed:unpleasant (1:0:0) |
| **Heroin at home** | **41/46** | **2/46** | **3/46** | p>0.05 | p>0.05 |
| **Heroin outside the home** | **18/46** | **5/46** | **23/46** | p<0.01 | p<0.01 |
| **Cocaine at home** | **14/52** | **6/52** | **32/52** | p<0.01 | p<0.01 |
| **Cocaine outside the home** | **26/52** | **9/52** | **17/52** | p<0.01 | p<0.01 |

Table 5. Shift in valence of heroin and cocaine subjective experience as a function of setting. The McNemar's Test indicated a significant shift in valence for both cocaine and heroin; *p<0.00001, #p=0.0014.

| | | Heroin outside the home | |
|---|---|---|---|
| | | Pleasant | Unpleasant |
| Heroin at home | Pleasant | 17 (no shift) | 26 (predicted shift)* |
| | Unpleasant | 1 (unpredicted shift) | 2 (no shift) |

| | | Cocaine outside the home | |
|---|---|---|---|
| | | Pleasant | Unpleasant |
| Cocaine at home | Pleasant | 9 (non shift) | 6 (unpredicted shift) |
| | Unpleasant | 24 (predicted shift)# | 11 (no shift) |

## 5. References


Antoniazzi, Raquel Pippi, Amanda Rodrigues Sari, Maísa Casarin, Cristina Machado Bragança de Moraes, and Carlos Alberto Feldens. 2017. "Association between Crack Cocaine Use and Reduced Salivary Flow." *Brazilian Oral Research* 31 (June): e42.

Avvisati, Riccardo, Laura Contu, Emiliana Stendardo, Caterina Michetti, Christian Montanari, Maria Luisa Scattoni, and Aldo Badiani. 2016. "Ultrasonic Vocalization in Rats Self-Administering Heroin and Cocaine in Different Settings: Evidence of Substance-Specific Interactions between Drug and Setting." *Psychopharmacology* 233 (8): 1501–11.

Badiani, Aldo. 2013. "Substance-Specific Environmental Influences on Drug Use and Drug Preference in Animals and Humans." *Current Opinion in Neurobiology* 23 (4): 588–96.

Badiani, Aldo, and Primavera A. Spagnolo. 2013. "Role of Environmental Factors in Cocaine Addiction." *Current Pharmaceutical Design* 19 (40): 6996–7008.

Berridge, Kent C. 2012. "From Prediction Error to Incentive Salience: Mesolimbic Computation of Reward Motivation." *The European Journal of Neuroscience* 35 (7): 1124–43.

Berridge, Kent C., and Morten L. Kringelbach. 2013. "Neuroscience of Affect: Brain Mechanisms of Pleasure and Displeasure." *Current Opinion in Neurobiology* 23 (3): 294–303.

Berridge, Kent C., Terry E. Robinson, and J. Wayne Aldridge. 2009. "Dissecting Components of Reward: 'Liking', 'Wanting', and Learning." *Current Opinion in Pharmacology* 9 (1): 65–73.

Billman, G. E. 1995. "Cocaine: A Review of Its Toxic Actions on Cardiac Function." *Critical Reviews in Toxicology* 25 (2): 113–32.

Cacioppo, J. T., R. E. Petty, M. E. Losch, and H. S. Kim. 1986. "Electromyographic Activity over Facial Muscle Regions Can Differentiate the Valence and Intensity of Affective Reactions." *Journal of Personality and Social Psychology* 50 (2): 260–68.

Camilleri, Michael. 2011. "Opioid-Induced Constipation: Challenges and Therapeutic Opportunities." *The American Journal of Gastroenterology* 106 (5): 835–42; quiz 843.

Caprioli, Daniele, Michele Celentano, Alessandro Dubla, Federica Lucantonio, Paolo Nencini, and Aldo Badiani. 2009. "Ambience and Drug Choice: Cocaine- and Heroin-Taking as a Function of Environmental Context in Humans and Rats." *Biological Psychiatry* 65 (10): 893–99.

Caprioli, Daniele, Michele Celentano, Giovanna Paolone, and Aldo Badiani. 2007. "Modeling the Role of Environment in Addiction." *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 31 (8): 1639–53.

Caprioli, Daniele, Michele Celentano, Giovanna Paolone, Federica Lucantonio, Andrea Bari, Paolo Nencini, and Aldo Badiani. 2008. "Opposite Environmental Regulation of Heroin and Amphetamine Self-Administration in the Rat." *Psychopharmacology* 198 (3): 395–404.

Caprioli, Daniele, Giovanna Paolone, Michele Celentano, Arianna Testa, Paolo Nencini, and Aldo Badiani. 2007. "Environmental Modulation of Cocaine Self-Administration in the Rat." *Psychopharmacology* 192 (3): 397–406.

Critchley, Hugo D. 2009. "Psychophysiology of Neural, Cognitive and Affective Integration: fMRI and Autonomic Indicants." *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology* 73 (2): 88–94.

Cochrane, C., R. Malcolm, and T. Brewerton. 1998. "The Role of Weight Control as a Motivation for Cocaine Abuse." *Addictive Behaviors* 23 (2): 201–7.

Cohen, Jacob. 1992. "Statistical Power Analysis." *Current Directions in Psychological Science* 1 (3). [Association for Psychological Science, Sage Publications, Inc.]: 98–101.

Craig, Arthur D. 2002. "Opinion: How Do You Feel? Interoception: The Sense of the Physiological Condition of the Body." *Nature Reviews. Neuroscience* 3 (8). Nature Publishing Group: 655.

Cunningham, E. E., R. C. Venuto, and M. A. Zielezny. 1984. "Adulterants in Heroin/cocaine: Implications Concerning Heroin-Associated Nephropathy." *Drug and Alcohol Dependence* 14 (1): 19–22.

Darke, S. 1998. "Self-Report among Injecting Drug Users: A Review." *Drug and Alcohol Dependence* 51 (3): 253–63; discussion 267–68.

Darwin, Charles. 1998. *The Expression of the Emotions in Man and Animals*. Oxford University Press.

Dearing, Ronda L., Jeffrey Stuewig, and June Price Tangney. 2005. "On the Importance of Distinguishing Shame from Guilt: Relations to Problematic Alcohol and Drug Use." *Addictive Behaviors* 30 (7): 1392–1404.

Ekman, P., R. W. Levenson, and W. V. Friesen. 1983. "Autonomic Nervous System Activity Distinguishes among Emotions." *Science* 221 (4616): 1208–10.

Ersche, Karen D., Jan Stochl, Jeremy M. Woodward, and Paul C. Fletcher. 2013. "The Skinny on Cocaine: Insights into Eating Behavior and Body Weight in Cocaine-Dependent Men." *Appetite* 71 (December): 75–80.

Ettenberg, Aaron. 2004. "Opponent Process Properties of Self-Administered Cocaine." *Neuroscience and Biobehavioral Reviews* 27 (8): 721–28.

Ettenberg 2009. "The Runway Model of Drug Self-Administration." *Pharmacology, Biochemistry, and Behavior* 91 (3): 271–77.

Gray, Marcus A., Felix D. Beacher, Ludovico Minati, Yoko Nagai, Andrew H. Kemp, Neil A. Harrison, and Hugo D. Critchley. 2012. "Emotional Appraisal Is Influenced by Cardiac Afferent Information." *Emotion* 12 (1): 180–91.

Goldstein, Rita Z., A. D. Bud Craig, Antoine Bechara, Hugh Garavan, Anna Rose Childress, Martin P. Paulus, and Nora D. Volkow. 2009. "The Neurocircuitry of Impaired Insight in Drug Addiction." *Trends in Cognitive Sciences* 13 (9): 372–80.

Haddad, G. G., and P. A. Lasala. 1987. "Effect of Parasympathetic Blockade on Ventilatory and Cardiac Depression Induced by Opioids." *Respiration Physiology* 67 (1): 101–14.

Hallman, David M., and Eugene Lyskov. 2012. "Autonomic Regulation in Musculoskeletal Pain." In *Pain in Perspective*. InTech.

Herbert, Beate M., Cornelia Herbert, Olga Pollatos, Katja Weimer, Paul Enck, Helene Sauer, and Stephan Zipfel. 2012. "Effects of Short-Term Food Deprivation on Interoceptive Awareness, Feelings and Autonomic Cardiac Activity." *Biological Psychology* 89 (1): 71–79.

Izard, C. E. 1992. "Basic Emotions, Relations among Emotions, and Emotion-Cognition Relations." *Psychological Review* 99 (3): 561–65.

James, William. 1884. "What Is an Emotion?" Mind; a Quarterly Review of Psychology and Philosophy 9 (34). *[Oxford University Press, Mind Association]:* 188–205.

Johnson, Matthew W., Evan S. Herrmann, Mary M. Sweeney, Robert S. LeComte, and Patrick S. Johnson. 2017. "Cocaine Administration Dose-Dependently Increases Sexual Desire and Decreases Condom Use Likelihood: The Role of Delay and Probability Discounting in Connecting Cocaine with HIV." *Psychopharmacology* 234 (4): 599–612.

Kaye, Linda K., Stephanie A. Malone, and Helen J. Wall. 2016. "Emojis: Insights, Affordances, and Possibilities for Psychological Science." *Trends in Cognitive Sciences*, November. doi:10.1016/j.tics.2016.10.007.

Köster, Egon P., and Jozina Mojet. 2015. "From Mood to Food and from Food to Mood: A Psychological Perspective on the Measurement of Food-Related Emotions in Consumer Research." *Food Research International* 76 (October): 180–91.

Kreibig, Sylvia D. 2010. "Autonomic Nervous System Activity in Emotion: A Review." *Biological Psychology* 84 (3): 394–421.

Lang, Gladys Engel, and Kurt Lang. 1983. *The Battle for Public Opinion: The President, the Press, and the Polls during Watergate*. Columbia UP.

Lang, P. J., M. K. Greenwald, M. M. Bradley, and A. O. Hamm. 1993. "Looking at Pictures: Affective, Facial, Visceral, and Behavioral Reactions." *Psychophysiology* 30 (3): 261–73.

Larsen, R. J., & Denier, E. 1992. "Promises and problems with the circumplex model of emotions. In M.S. Clark (Ed.), Emotions (pp. 25-29). Beverly Hills, CA: Sage

Larson, Merlin D. 2008. "Mechanism of Opioid-Induced Pupillary Effects." *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology* 119 (6): 1358–64.

Levenson, Robert W. 2014. "The Autonomic Nervous System and Emotion." *Emotion Review: Journal of the International Society for Research on Emotion* 6 (2). SAGE Publications: 100–112.

Luoma, Jason B., Barbara S. Kohlenberg, Steven C. Hayes, and Lindsay Fletcher. 2012. "Slow and Steady Wins the Race: A Randomized Clinical Trial of Acceptance and Commitment Therapy Targeting Shame in Substance Use Disorders." *Journal of Consulting and Clinical Psychology* 80 (1): 43–53.

Luoma, Jason B., Richard H. Nobles, Chad E. Drake, Steven C. Hayes, Alyssa O'Hair, Lindsay Fletcher, and Barbara S. Kohlenberg. 2013. "Self-Stigma in Substance Abuse: Development of a New Measure." *Journal of Psychopathology and Behavioral Assessment* 35 (2): 223–34.

Maceira, Alicia M., Carmen Ripoll, Juan Cosin-Sales, Begoña Igual, Mirella Gavilan, Jose Salazar, Vicente Belloch, and Dudley J. Pennell. 2014. "Long Term Effects of Cocaine on the Heart Assessed by Cardiovascular Magnetic Resonance at 3T."

*Journal of Cardiovascular Magnetic Resonance: Official Journal of the Society for Cardiovascular Magnetic Resonance* 16 (April): 26.

Massey, Frank J. 1951. "The Kolmogorov-Smirnov Test for Goodness of Fit." *Journal of the American Statistical Association* 46 (253). [American Statistical Association, Taylor & Francis, Ltd.]: 68–78.

McNemar, Q. 1947. "Note on the Sampling Error of the Difference between Correlated Proportions or Percentages." *Psychometrika* 12 (2): 153–57.

Nathanson Lori, Susan E. Rivers, Lisa M. Flynn, Marc A. 2016. "Brackett Creating Emotionally Intelligent Schools With RULER." *Emotion Review* 8(4): 305-10.

Nilsson, Matias, Jakob L. Poulsen, Christina Brock, Thomas H. Sandberg, Mikkel Gram, Jens B. Frøkjær, Klaus Krogh, and Asbjørn M. Drewes. 2016. "Opioid-Induced Bowel Dysfunction in Healthy Volunteers Assessed with Questionnaires and Mri." *European Journal of Gastroenterology & Hepatology* 28 (5). European Journal of Gastroenterology & Hepatology: 514–24.

Noël, Xavier, Damien Brevers, and Antoine Bechara. 2013. "A Neurocognitive Approach to Understanding the Neurobiology of Addiction." *Current Opinion in Neurobiology* 23 (4): 632–38.

Paulus, Martin P., and Jennifer L. Stewart. 2014. "Interoception and Drug Addiction." *Neuropharmacology* 76 Pt B (January): 342–50.

Paulus, Martin P., Susan F. Tapert, and Gery Schulteis. 2009. "The Role of Interoception and Alliesthesia in Addiction." *Pharmacology, Biochemistry, and Behavior* 94 (1): 1–7.

Posner, Jonathan, James A. Russell, and Bradley S. Peterson. 2005. "The Circumplex Model of Affect: An Integrative Approach to Affective Neuroscience, Cognitive Development, and Psychopathology." *Development and Psychopathology* 17 (3): 715–34.

Price, Cynthia, and Kathleen Smith-DiJulio. 2016. "Interoceptive Awareness Is Important for Relapse Prevention: Perceptions of Women Who Received Mindful Body Awareness in Substance Use Disorder Treatment." *Journal of Addictions Nursing* 27 (1): 32–38.

Redan, Jay A., Tina Wells, Sandra Reeder, and Steven D. McCarus. 2016. "Reducing Opioid Adverse Events: A Safe Way to Improve Outcomes." *Surgical Technology International* 28 (April): 101–9.

Russell, J. A. 1983. "Pancultural Aspects of the Human Conceptual Organization of Emotions." *Journal of Personality and Social Psychology*. psycnet.apa.org. http://psycnet.apa.org/psycinfo/1984-14108-001.

Russell, J. A., and B. Fehr. 1994. "Fuzzy Concepts in a Fuzzy Hierarchy: Varieties of Anger." *Journal of Personality and Social Psychology* 67 (2): 186–205.

Russell, James. 2003. "Introduction: The Return of Pleasure." *Cognition and Emotion* 17 (2). Routledge: 161–65.

Russell, James A. 1980. "A Circumplex Model of Affect." *Journal of Personality and Social Psychology* 39 (6). American Psychological Association: 1161.

Saarni, Carolyn. 1999. *The Development of Emotional Competence*. Guilford Press.

Scherer, Klaus R. 2001. "Appraisal Considered as a Process of Multilevel Sequential Checking." *Appraisal Processes in Emotion: Theory, Methods, Research* 92 (120). New

York, NY: 57.

Scherer, Klaus R. 2005. "What Are Emotions? And How Can They Be Measured?" *Social Sciences Information. Information Sur Les Sciences Sociales* 44 (4): 695–729.

Schlosberg, H. 1952. "The Description of Facial Expressions in Terms of Two Dimensions." *Journal of Experimental Psychology* 44 (4): 229–37.

Sherrington, Charles. 1948 "The Integrative Action of the Nervous System". Cambridge Univeristy Press, Cambridge, UK (1948).

Skinner, H. A., and B. A. Allen. 1983. "Does the Computer Make a Difference? Computerized versus Face-to-Face versus Self-Report Assessment of Alcohol, Drug, and Tobacco Use." *Journal of Consulting and Clinical Psychology* 51 (2): 267–75.

Smith, Kyle S., Kent C. Berridge, and J. Wayne Aldridge. 2011. "Disentangling Pleasure from Incentive Salience and Learning Signals in Brain Reward Circuitry." *Proceedings of the National Academy of Sciences of the United States of America* 108 (27): E255–64.

Sofuoglu, Mehmet, and R. Andrew Sewell. 2009. "Norepinephrine and Stimulant Addiction." *Addiction Biology* 14 (2): 119–29.

Team, R. C. 2014. "R: A Language and Environment for Statistical Computing.. R Foundation for Statistical Computing, Vienna, Austria. URL Http://www. R-Project. org/(Date of …." Accessed 01/12.

Thornhill, J. A., C. Townsend, and L. Gregor. 1989. "Intravenous Morphine Infusion (IMF) to Drug-Naive, Conscious Rats Evokes Bradycardic, Hypotensive Effects, but Pressor Actions Are Elicited after IMF to Rats Previously given Morphine." *Canadian Journal of Physiology and Pharmacology* 67 (3): 213–22.

Watson, D., D. Wiese, and J. Vaidya. 1999. "The Two General Activation Systems of Affect: Structural Findings, Evolutionary Considerations, and Psychobiological Evidence." *Journal of Personality*. psycnet.apa.org. doi:10.1037//0022-3514.76.5.820.

Winkielman, Piotr, and Kent Berridge. 2003. "Irrational Wanting and Subrational Liking: How Rudimentary Motivational and Affective Processes Shape Preferences and Choices." *Political Psychology* 24 (4). Blackwell Publishing Inc: 657–80.

Wise, R. A. 1980. "Action of Drugs of Abuse on Brain Reward Systems." *Pharmacology, Biochemistry, and Behavior* 13 Suppl 1: 213–23.

Wise, Roy A. 2008. "Dopamine and Reward: The Anhedonia Hypothesis 30 Years on." *Neurotoxicity Research* 14 (2-3): 169–83.

Wundt, Wilhelm. 1874. "1905." *Grundzüuge Der Physiologischen Psychologie,[Fundamentals of Physiological Psychology, Orig. Pub. 1874] Fifth Ed. Engelmann, Leipzig*.

Zajonc, Robert B. 1980. "Feeling and Thinking: Preferences Need No Inferences." *The American Psychologist* 35 (2). American Psychological Association: 151.

# Chapter 3

## An fMRI study of heroin- versus cocaine-related imagery in human addicts: the role of setting

### 1. Introduction

The subjective and behavioural effects of addictive drugs are not a simple consequence of their primary neuropharmacological actions. It has long been pointed out that contextual variables play an important in shaping the subjective effects of drugs (Zinberg 1984). Our understanding of the nature of contextual influences on drug reward is still very limited. So far, most preclinical and clinical research has focused on the role of stress and of drug cues (see Caprioli et al. 2007). However, we have recently reported that the setting of drug taking can affect drug reward in a substance-specific manner and that it does so in a way that is not easily reducible to stress or conditioning. Intravenous self-administration (SA) experiments in the rat, for example, have shown that the relative preference for heroin vs. cocaine varies as a function of the setting. The rewarding effects of heroin appear to be greater in rats that reside in the self-administration environment (which is therefore also their home environment) than in rats that do not reside in the self-administration chamber (which is an environment distinct from the home cage), whereas the opposite is observed for the rewarding effects of cocaine (which was greater in non resident rats than in resident rats). This relatively simple manipulation of the setting of drug taking exerts a substance-specific influence on all aspects of cocaine vs. heroin reward, including drug intake, motivation to work for the

drug (Caprioli et al. 2007b; 2008), drug discrimination (Paolone et al. 2004; Caprioli et al. 2007b), drug affect (Avvisati et al. 2016), drug choice (Caprioli et al. 2009), and vulnerability to relapse into cocaine or heroin seeking after a period of abstinence in an animal model of relapse (Montanari et al. 2015).

Translational studies conducted in addicts have shown that even in humans drug and setting interact in a manner reminiscent to that observed in rats, even if the experimental design was necessarily different (Caprioli et al. 2009; Badiani and Spagnolo 2013). Heroin and cocaine co-abusers reported in fact to prefer different settings for cocaine versus heroin. Most addicts reported using heroin prevalently at home and cocaine prevalently outside the home. Location preferences reflected real preferences rather than social or practical considerations, as indicated by the fact that similar results were observed for both solitary and social use and for all routes of drug taking.

The main aim of this study was to begin an investigation of the neural basis of the interaction between drug and setting in humans. In situ hybridization and immunohistochemistry studies in rats have shown that the pattern of neuronal activation (as indicated by the expression of Fos and Fos mRNA) in response to addictive drugs such as amphetamine, cocaine, heroin, and morphine is very different in rats taking the drug at home relative to rats taking the drug outside the home (Badiani et al. 1998, 1999; Ferguson and Robinson 2004; Hope et al. 2006; Paolone et al. 2007; Celentano et al. 2009). These differences were particularly evident in the striatal complex and in the Prefrontal Cortex (PFCx), two regions implicated in drug reward in both rodents and humans (Breiter et al. 1997; Cox et al. 2009; Kufahl et al. 2005; Leyton and Vezina 2013; Risinger et al. 2005; Volkow et al. 1999; Watson et al. 2014). In the present study, we used emotional imagery and 3T functional

Magnetic Resonance Imaging (fMRI) to investigate drug and setting interaction in addicts with a history of heroin and cocaine abuse. Functional MRI methodology detects the magnetic properties of oxygenated *vs* deoxygenated haemoglobin and transforms it into an indirect measure of neural activity. The signal detected is also referred to as the blood oxygen level dependent (BOLD) signal.

Our everyday perception of the environment reflects the interaction between exteroceptive and interoceptive information. However, even in the absence of bottom-up signals it is still possible to generate internal visual representations using top-down signals only, commonly referred to as mental imagery (Lee et al. 2012). A growing body of literature indicates that mental imagery involves high-level cognitive functions such as perception, memory, emotion, and motor control (Rollins 1992; Kosslyn, Ganis, and Thompson 2001; Owen et al. 2006; Berger and Ehrsson 2014). Previous studies have reported similar neural substrates for imagery and perception (Ishai and Sagi 1995; Ganis, Thompson, and Kosslyn 2004). However, imagery is often seen as a "weak version of perception" (Lee, Kravitz, and Baker 2012). There is evidence suggesting that despite the similarities, imagery and perception represent two distinct functions. For example, studies on patients with lesions revealed that there is a double dissociation between the two functions, with some patients exhibiting preserved imagery despite an impaired perception and vice versa (Behrmann, Winocur, and Moscovitch 1992; Behrmann, Moscovitch, and Winocur 1994; Lee, Kravitz, and Baker 2012).

Hence, mental imagery seems to represent a reliable method to investigate virtual drug and setting interactions in real-time using the fMRI technique. We, therefore, adapted an emotional imagery procedure based on

previous work by Lang and colleagues (Lang 1979; Lang et al. 1980) to recreate two different real-world settings of drug use and asked the participants to imagine taking heroin and cocaine. We hypothesized a double dissociation of the BOLD signal in the brain regions implicated in drug reward, such as the prefrontal cortex and the striatum.

## 2. Material and Methods

### 2.1 Participants

Twenty male addicts (aged 35.35±8.13 years) with history of heroin (13.20±6.29 years) and cocaine (15.25±5.74 years) abuse were recruited among the outpatients of the Substance Misuse Services of Villa Maraini (Rome, Italy). Participants were enrolled at the moment of their daily visit by the medical staff and social operators of Villa Maraini and underwent a comprehensive diagnostic interview. Criteria for inclusion in the study were: 1) drug dependence criteria for cocaine and/or heroin (DSM-IV-R); 2) no other major psychiatric disorder (DSM-IV-R); 3) fixed address; 4) heroin and/or cocaine use at least once/week in the past 12 months; 5) no history of neurological disorder or head trauma with loss of consciousness exceeding 30 min; and 6) have no contraindications to MRI. Individuals with alcohol use requiring medical detoxification were excluded. Nineteen participants were enrolled in methadone replacement programs (mean±SD dose = 39.47±29.62). Additional demographic information is reported in Table 1.

All procedures were approved by the IRCSS Santa Lucia Foundation Ethics Committee and carried out in accordance with the Declaration of

Helsinki. The participants were legally competent and did not exhibit a compromised ability/capacity to provide informed written consent. The participants received a flat reimbursement of 20 euro for their time. Refreshments were provided during the pre and post-scanning time.

## 2.2. Emotional Imagery procedures

### *2.2.1. Scripts*

The scripts for the imagery task were developed based on self-reports collected in occasion of previous studies (Caprioli et al. 2009; Badiani and Spagnolo 2013) and on the basis of pilot interviews with individuals satisfying the inclusion criteria used in the present study.

During mental imagery, perceptual representations can be evoked in the absence of external sensory input. Previous studies have reported similar neural substrates for imagery and perception demonstrating that it is possible to induce a subjective experience resembling an actual perceptual experience by using verbal instructions based on an appropriate script and instructions (Lang 1979; Reddy et al. 2010; Cichy et al. 2012; Lee et al. 2012).

In particular, it appears that emotional imagery procedures are capable of eliciting a mental representation that is not simply a picture scanned with the "mind's eye" but a dynamic scenario based on real-life experience. In agreement with previous emotional imagery studies (Lang 1979; Lang et al. 1980; Costa et al. 2010; Cuthbert et al. 2003; Dougherty et al. 1999; McTeague et al. 2009), each script was structured to include:

1. The instruction to create a mental image;

2. The description of the scenario to be imagined. In this case, one of two naturalistic settings: the participant's own home (*'home'* condition) and the participant's habitual club (*'outside-the-home'* condition);
3. The instruction to imagine oneself engaging in heroin or cocaine use (at home or outside the home) '*as if*' it were really happening.

Thus, we created two scripts for the baseline imagery task, in which participants were asked to visualize themselves relaxing at home or in their usual club, and four scripts for the drug imagery task, one script for each combination of drug and setting: i) cocaine at home, ii) cocaine outside the home, iii) heroin at home, iv) heroin outside the home. The scripts did not include any information specific to the individual; that is, the scripts had a standard format that was applicable to all subjects (Table 2). The scripts were then recorded and played during the imagery tasks.

*2.2.2. Imagery training session*

A week prior to the fMRI session, the subjects underwent an imagery training session conducted at the addiction clinic "Villa Maraini". The main aim of the session was to familiarize the participants with the imagery procedure while listening to a recording of the scanner noise through headphones. Previous studies have shown that imagery training can also increase the emotional response during the imagery task (Miller et al. 1987; Sinha 2009).

The participants were asked to complete the following imagery questionnaires (adapted to Italian by Antonietti and Crespi, unpublished manuscript): 1) Vividness of Visual Imagery Questionnaire (VVIQ; Marks 1989) in which the participants were instructed to visualize themselves in standard environmental contexts; 2) Vividness of Movements Imagery Questionnaire

(VMIQ; Isaac et al. 1986) in which participants were instructed to visualize themselves while performing specific movements; 3) Test of Visual Imagery Control (TVIC; Gordon 1949), in which the participants were instructed to operate a transformation on a mental image; 4) An adaptation of the Questionnaire on "Imagery induction of emotional state" (Wright and Mischel 1982), in which participants were instructed to imagine situations associated with a 'positive' emotional state (serene, happy, surprised and relaxed) or to a 'negative' emotional state (hungry, fearful, disgusted, sad). The participants were instructed to close their eyes, to ignore as far as possible the noise of the scanner, to create a mental scenario for each condition (based on hypothetical or real events from their personal life), to imagine 'living' this scenario, and finally to rate the vividness of the imagery by assigning a score from 1 (not clear at all) to 5 (perfectly clear) to each item in the questionnaires. The imagery training session lasted approximately 40 min. Participants with a performance of less 25% of the maximum total score for two imagery scales, or who exhibited sign of distress due to the noise of the scanner were excluded from the study.

**2.3 fMRI procedures**

On the day of the fMRI scan, participants underwent a urine drug screen for amphetamine, barbiturates, benzodiazepines, cocaine, methadone, opiates, methamphetamine, and THC at the addiction clinic Villa Maraini. They were then transferred to the Brain Imaging facility of the Santa Lucia Foundation. Sixteen participants took their usual dose of methadone before the leaving the Clinic. Participants who smoked were allowed to smoke prior to the scan.

All the participants were blind about the content of the experimental session. Just before the fMRI session, participants were instructed to complete the imagery task using their own personal experience with heroin and cocaine but were asked to exclude instances of combined heroin and cocaine use ('speedball').

Before entering the fMRI scanner, the participants received the following instructions by the experimenter:

> *"You will be asked to imagine yourself in two different settings, specifically to be either at your own home or in your usual club. Your task will be to visualize as vividly as possible the setting in your mind and to focus intensely on this situation as if it were really happening at that moment. You will then be asked to imagine to use heroin or cocaine in that very same setting. You should try to focus on the effects produced by the drug while in that specific setting.*
>
> *When asked to imagine being at home it is really important that it is your own home. You can imagine being in any part of your home (living room, bedroom, bathroom, kitchen) where you usually take or have taken the drug. If you have never taken that drug in that setting, try to imagine how it would be actually to do so. The imagined event should take place in the evening, at 21:00 hours.*
>
> *When asked to imagine being in a club, it is really important that it is, or has been, your usual club. You can imagine being in any part of the club where you usually take or have taken the drug. If you have never taken that drug in that setting, try to imagine how it would be to actually to do so. The imagined event should take place in the evening, at 21:00 hours."*

The study design is outlined in Figure 1A. During the fMRI session, each participant underwent a total of eight scans, two for each combination of drug and setting (i.e., heroin at home, cocaine at home, heroin outside, cocaine outside), in a pseudo-random sequence, counterbalanced across subjects.

Subjects were equipped with headphones, and each scan was preceded by audio and video instructions guiding them through the imagery task.

Each trial consisted of 60 s baseline imagery (relaxing at home or outside the home), followed by 120 s of drug imagery, and 60 s of rest in which subjects were asked to stop imagery (see Figure 1). Immediately after the end of each trial, the participants rated the vividness of the imagery on using a VAS (visual analogue scale) ranging from 1 ('not vivid at all') to 10 ('perfectly vivid') displayed on a screen, using a push button controller.

Rating of craving intensity and pleasure were collected at the end of the fMRI session using the following VASs:

Craving: *"Please rate the intensity of your desire to use heroin/cocaine at the moment"*; with the anchors 1 ('absent') and 10 ('extremely high').

Pleasure: "*Please rate the level of pleasure experienced in each of the following conditions: i) taking heroin at home; ii) taking cocaine at home; iii) taking heroin outside the home; iv) taking cocaine outside the home"*; with the anchors 1 ('non pleasurable at all') and 10 ('extremely pleasurable').

Finally, we asked the participants to rank the four conditions based on the level of pleasure, from 1 (most pleasurable) to 4 (least pleasurable)

**2.4 Apparatus and image acquisition**

Functional MRI runs sensitive to blood oxygenation level-dependent (BOLD) contrast were collected in a block-design using a Siemens Allegra scanner (Siemens Medical Systems, Erlangen, Germany) 3.0 Tesla scanner operating at the Neuroimaging Laboratory, Foundation Santa Lucia. Stimuli were generated by a control computer located outside the MR room, running an in-house software implemented in MatLab (Galati et al., 2008, Sulpizio et al. 2013, Boccia

et al., 2014). Instructions were presented simultaneously in audio and video modalities. An LCD video was used to project instructions to a back projector screen mounted inside the MR tube and visible through a mirror located inside the head coil. Presentation timing was synchronized by the acquisition of fMRI images. Responses were given through push button connected to the computer by optic fibers.

Head movements were minimized by mild restraint and cushioning. Functional MRI images were acquired for the entire brain using a gradient echo planar imaging (EPI) sequence covering the whole brain (34 slices, in-plane resolution=3x3 mm, slice thickness=3 mm, inter-slice distance=1.25 mm, repetition time [TR]=2210 ms, echo time [TE]=30 ms, flip angle=70° deg). For each scan, a total of 113 fMRI volumes were acquired. High-resolution 3D T1-weighted MRI scan was acquired for each subject using a magnetization-prepared rapid gradient echo sequence (Siemens MPRAGE, 176 slices, in-plane resolution= 0.5 mm, in-plane resolution=0.5x0.5 mm, slice thickness=1 mm, TR=2000 ms, TE=4.38 ms, flip angle=8° deg).

**2.5. Image analysis**

Image analyses were performed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK http://www.fil.ion.ucl.ac.uk/spm) implemented in MatLab (MATLAB 2011a, The MathWorks, Inc., Natick, MA, USA). The first four volumes of each scan were discarded to allow for T1 equilibration effects.

Functional time series from each subject, were temporally corrected for slice timing, using the middle volume in time as a reference, and then spatially corrected for head movement (realigned), using a least-squares approach and

six parameters rigid body spatial transformation. The images were then coregistered onto their T1 image and spatially normalized using an automatic nonlinear stereotaxic normalization procedure (final voxel size: 3x3x3 mm) and spatially smoothed with a three dimensional Gaussian filter (6 mm full-width-half-maximum (FWHM). The template image for spatial normalization into a standard stereotaxic space was based on Montreal Neurological Institute (MNI-152) EPI template and conform to a standard coordinate referencing system (Talairach and Tournoux 1988).

Images were analyzed using a standard random-effect procedure. The time series of functional MR images obtained from each participant were analyzed separately. The effect of the experimental paradigm was estimated on a voxel by voxel basis, according to the general linear model extend to allow the analysis of fMRI data as time series. The model included a temporal high-pass filter to remove low frequency confounds with a period >128 s. Serial correlations in the fMRI time series were estimated with a restricted maximum likelihood (ReML) algorithm assuming the same correlation structure for each voxel, within each scan. The ReML estimates were then used to whiten the data.

Initially, neural activation during the imagery task was modeled as a box-car function spanning the whole duration of the imagery period and convolved with a canonical haemodynamic response function (HRF), chosen to represent the relationship between neuronal activation and blood oxygenation (Friston et al. 1998). Images of subject-specific parameter estimates, which represented activation relative to the baseline, were calculated for each of the four drug imagery scenarios and compared to the respective baseline imagery. The resting period was excluded from the data analysis due to potential carryover effect of the imagery period.

Using an "*omnibus*" F-contrast, we searched for voxels exhibiting differences in the BOLD signal between the baseline and the imagery task irrespective of the specific drug-setting combination. The resulting statistical parametric map was corrected for multiple comparisons based on family-wise error (FWE) at cluster level $p$=0.05, and is shown on Figure 2. These initial analyses aimed at selecting a map of regions implicated in the drug imagery task.

To avoid the risk of circularity (Kriegeskorte et al. 2009), we adopted an exploratory (rather than hypothesis-driven) approach to the identifications of the ROIs. That is, the regions identified based on this functional map (see Table 3) were further analyzed by creating a Region of Interest (ROI) from each maxima in the cluster analysis and by grouping together all neighboring voxels at a maximum distance of 16mm from the peak (Poldrack 2007). We computed a regional estimate of the amplitude of the haemodynamic response in each experimental condition by entering a spatial average (across all voxels in that region) of the pre-processed time series into the GLM.

The modulation of drug and setting on the signal from the resulting brain regions was then estimated by applying a deconvolution approach to the regionally averaged time courses from each region. We modelled each trial as a set of twelve finite impulse response (FIR) basis functions (Burock and Dale 2000; Ollinger et al. 2001) spanning 10 s each, starting from the onset of the imagery task. Such approach allows for a flexible haemodynamic response function (HRF) modelling without any assumption on the shape of the hemodynamic response in the time period within each trial where the difference in the signal arose, although remaining in the General Linear Model (GLM) framework (Steffener et al. 2010).

To directly examine the interactions relevant to our experimental questions, the resulting regional hemodynamic responses were analysed using 3-way analysis of variance (ANOVA) with repeated measures on the factors drug (2 levels: heroin vs. cocaine), setting (2 levels: home vs. outside the home) and time (12 levels).

## 3. Results

### 3.1. fMRI

#### *3.1.1. Whole brain analyses*

Whole-brain effects of the drug imagery task compared to the baseline is provided in Figure 2 (see also Table 3).

The general picture of the drug related imagery as a function of different context, show significantly activated different regions (specifically dominant in the left hemisphere) in six regional clusters of the prefrontal cortex (PFCx), [Broadman Area (BA) 6, 8, 44, 45, 46], the supplementary motor area (BA 32), the insula, the angular gyrus, the posterior cingulum and the precuneus. Bilateral activations were present at the level of the caudate, thalamus, brain stem, inferior temporal gyrus, and cerebellum.

#### *3.1.2. FIR analysis*

The changes in BOLD signal during drug imagery followed three distinct temporal patterns.

Some regions of the left PFCx, Caudate and Cerebellum exhibited a double dissociation in BOLD signal. The change in BOLD signal was, in fact, greater when the addicts were asked to imagine taking heroin outside the home and cocaine at home (the less preferred settings), compared to heroin at home and cocaine outside the home (the preferred settings). Three of these clusters were located in the inferior and middle frontal gyrus of the left PFCx: BA44 ($F_{(1,19)}$=10.72; $p$=0.004), BA 8, ($F_{(1,19)}$=4.88; $p$=0.04), and BA 46 ($F_{(1,19)}$=7.05; $p$=0.016) (Figure 3). Other clusters were located in the left caudate ($F_{(1,19)}$=4.20, $p$=0.05), in Crus I and II of the left ($F_{(1,19)}$=6.45, $p$=0.02) and right ($F_{(1,19)}$=7.9, $p$=0.01) cerebellum (Figure 4), and in the right brain stem ($F_{(1,19)}$=5.52, $p$=0.03).

Furthermore, in these regions there was also a drug x setting x time interaction: BA 44 ($F_{(11,209)=}$5.45; $p$<0.0001), BA 8, ($F_{(11,209)=}$2.20; $p$=0.015), BA 46 ($F_{(11,209)}$=71.78; $p$=0.058), left caudate ($F_{(11,209)=}$3.92, $p$<0.0001), left cerebellum ($F_{(11,209)=}$3.38, $p$<0.0001), right cerebellum ($F_{(11,209)=}$5.81, $p$<0.0001), right brain stem ($F_{(11,209)=}$2.02, $p$=0.028).

In other areas brain areas such as the left angular gyrus, there was no such dissociation, and the change in BOLD signal was greater in the 'home' compared to the 'outside the home' condition, for both heroin and cocaine showing a significant main effect of the setting ($F_{(1,19)}$=7.15, $p$=0.015). The opposite pattern was found in the left supplementary motor area (BA 32), where the change in BOLD signal was in fact greater in the 'outside the home' than in the 'at home' condition for both drugs, although there was not a significant main effect of the setting ($F_{(1,19)}$=3.2, $p$=0.09).

The functional map shown no main effect the drug, apart from a marginally significant difference in the left precuneus, ($F_{(1,19)}$=3.82, $p$=0.06) with cocaine showing greater activation compared to heroin.

In all the other regions, there were no significant differences in BOLD signal as a function of drug or setting, and there were no interactions.

### 3.2. Subjective measures

#### *3.2.1. Imagery Vividness*

A non-parametric Friedman test among shown there were no significant differences in subjective vividness among the four drug imagery conditions $\chi^2$=3.30, $p$=0.3 (Figure 1b).

#### *3.2.2. Subjective Pleasure*

No significant differences in the ratings of subjective pleasure among the four conditions were found at the end of the fMRI task ($\chi^2$=1.46, $p$=0.7) ~~(Figure 6a).~~ However, when we asked the participants to rank the conditions for subjective pleasure (from 1 = *most pleasurable* to 4 = *least pleasurable*) the Test of Friedman indicated significant differences among conditions ($\chi^2$=20.46, $p$=0.001). As predicted, heroin at home ranked significantly higher (1.6±018) than heroin outside the home (3±0.20) (Wilcoxon Signed-Rank Test, one-tailed, $Z$=-1.88, $p$=0.03, $r$=0.4). In contrast, there were no significant differences between cocaine at home (3.0±0.15) and cocaine outside the home (3.2±0.22), $Z$=-0.66, $p$= 0.2, $r$=0.1 (Figure 6, bottom panel).

*3.2.3. Heroin and Cocaine Craving*

A two-tailed Wilcoxon signed rank test for paired data was conducted to determine whether there was a difference in the craving for heroin and cocaine pre and post-fMRI. As illustrated in Figure 6 (top panel), craving pre-fMRI for heroin was significantly higher ($M$=5.43, $SEM$=0.67) compared to cocaine ($M$=3.28, $SEM$=0.63), $Z$=1.93, $p$=0.05, $r$=0.4. However, the difference between the rating for the two drugs post-fMRI shown no significant difference (heroin: $M$=4.85, $SEM$=0.86; cocaine: $M$=4.35, $SEM$=0.67), $Z$=0.45, $p$=0.6, $r$=0.1. Cocaine craving showed a significant increase pre and post-scanner, $Z$=2.26, $p$=0.02, $r$=0.5, while no significant effects were reported for heroin craving, $Z$=0.74, $p$=0.4, $r$=0.2.

## 4. Discussion

The present study yielded two major novel findings. First, we found that cocaine- and heroin-related imagery in addicts induced distinct patterns of activation in the prefrontal cortex, caudate, and cerebellum. Second, the setting of drug taking influenced in opposite direction the effects of cocaine- versus heroin-related imagery on the activity of these brain regions.

Our working hypothesis predicted was that the setting should alter in opposite directions the effects of heroin and cocaine in at least some of the brain areas implicated in brain reward. Not only did our results confirm our prediction, but more surprisingly we found the same pattern of dissociation also in the cerebellum. Another surprising finding was represent by directionality of BOLD signal changes.

### 4.1. BOLD signal changes in the PFCx and Caudate

As illustrated in Figure 2, a limited number of cortical and subcortical regions were activated during the drug imagery tasks, including PFCx (BA6, BA8, BA44, BA45, BA46), Inferior Parietal cortex (BA40), Precuneus (BA7), Angular gyrus, Supplementary Motor area (BA32), Temporal gyrus (BA21), Posterior Cingulum, Caudate, Thalamus, Cerebellum (Crus I and Crus II), and Brain stem. However, only three regions we found an interaction between drug and setting: PFCx, Caudate, and Cerebellum.

Previous fMRI studies have shown an involvement of the PFCx and the Striatum in encoding drug reward (Goldstein and Volkow 2002; Cox et al. 2009; Volkow et al. 2012; Leyton and Vezina 2013; Goldstein and Volkow 2011). Thus, it was not surprising that the pattern of BOLD signal changes in these regions

during the imagery task exhibited the same dissociation in the interaction between drug and setting previously reported for a number of reward related measures in both humans and animals. Specifically, the two settings of drug use investigated here and in other studies (home environment versus non home environment) have been shown to influence in *opposite direction* the behavioural and subjective response to cocaine versus heroin (see Chapter 1 and 2). Thus, we did predict that also the changes BOLD signal in the PFCx and the Striatum in response to cocaine versus heroin imagery would be affected in opposite direction by the two settings.

A somewhat interesting aspect of our results is that portion of the Striatum most involved in the interaction was the dorsal Caudate and not the ventral Striatum (NAcc). (It must be noted, however, that the exact pattern of brain activation in brain imaging studies is also a function of the thresholds used in the statistical elaboration of the raw data. Thus, it is not possible to exclude that if we had pre-selected the ventral Striatum as a region of interest, also this area would have exhibited a pattern of activation similar to that observed in the Caudate.) Previous imaging studies in addicts have reported a selective involvement of the dorsal versus ventral striatum (Boileau et al. 2007; Volkow al. 2006; Wong, et al. 2006). A possible explanation for the selective involvement of the Caudate in the present study may derive from theoretical models that posit a shift in the processing of drug reward from the ventral to the dorsal Striatum with the development of 'habits' after extensive drug use (Everitt & Robbins 2016). However, a recent PET study with [11C]raclopride has shown that the dorsal caudate is selectively activated by drug cues even in after relatively little cocaine use, that is prior to the onset of addiction (Cox et al. 2017).

### 4.2. BOLD signal changes in the Cerebellum

A most interesting finding of the present study is represented by the bilateral involvement of the Cerebellum in processing drug setting interactions. There is now growing evidence that also the cerebellum is involved in processing drug reward and might play a role in drug addiction (for a review see Moulton et al. 2014; Miquel et al. 2009). In the past two decades, the traditional view of the cerebellum as primarily a motor structure has undergone a major upheaval based on increasing evidence indicating that it plays a pivotal role in modulating affective processes such as: i) emotional perception and encoding, ii) evaluation of emotional contexts, of bodily and facial expressions, of social interactions, and iii) regulation of emotional states in relation to motor, cognitive, and context-dependent tasks (Schmahmann 1996; Schmahmann and Sherman 1998; Schmahmann 2004; Scheuerecker et al. 2007; Stoodley 2012; Buckner 2013; Adamaszek et al. 2014; Van Overwalle et al. 2015; Adamaszek et al. 2017). Schmahmann and Sherman (1998) first coined the term "cerebellar cognitive affective syndrome" (CCAS), after observing in patients with focal cerebellar lesions a consistent pattern of impaired affect and cognitive ability (Schmahmann 1991; Schmahmann 2004). Cerebellar-dependant behavioural and emotional disorders have been conceptualized "as either excessive or reduced responses to the external or internal environment" (Schmahmann et al. 2007).

In brief, anatomically, the cerebellum receives sensory input from the spinal cord and integrates them with cortical input from prefrontal and association cortices to execute motor tasks (Ivry 1997; Kelly and Strick 2003; Ito 2006; Buckner 2013). Resting state fMRI (rs-fMRI) studies, examining the

functional connectivity (FC) of intrinsic brain networks have shown that the role of the cerebellum in a variety of functional resting state networks (RSN) associated with cortical area, far beyond motor areas (Habas et al. 2009; Buckner et al. 2011; Dobromyslin et al. 2012; Liu et al. 2017; Shinn et al. 2017). For example, it has been shown that there is an association between the cerebellar areas Crus I, with RSNs related to executive and associative processing and with brain regions involved in cognitive control, such as the dorsolateral PFCx and the dorsomedial PFCx (Buckner et al. 2011). Furthermore, other studies revealed an association between Crus I with an RSN that included the anterior insula and anterior cingulate cortex, which are areas involved in processing of stimulus salience (Habas et al. 2009) and interoception (Craig 2002; Paulus and Stewart 2014).

Emotional processing stem from subcortical networks that influence cortical activity. In particular, it has been suggested that two discrete and distinct neural substrates subserve emotional processing: one operating at an explicit level and the other at an implicit level, with the cerebellum, generally being considered as part of the network associated with implicit processing (Scheuerecker et al. 2007). Positive and negative emotions have been both considered to be processed by the cerebellar circuits (Turner et al. 2007; Baumann and Mattingley 2012), although a predominance of negative emotions processing has been suggested (Park et al. 2010; Ferrucci et al. 2012; Lupo et al. 2015). Recent neuroimaging evidences has shown that noxious heat and the passive viewing of unpleasant pictures activated the same regions in the cerebellum. This functional overlap between different unpleasant sensory modalities suggests that the cerebellum might be specifically involved in encoding aversive process. Thus, the activation of the cerebellar areas was

interpreted from the authors as reflecting aversion rather than stimulus salience (Moulton et al. 2011).

These results suggest that responses in these cerebellar regions may reflect multi-modal aversive processing which is not specific to pain processing, but also apply to other aversive sensory and affective experiences (Diano et al. 2016). An rCBF PET study that induced craving for cocaine found a response in the left posterior cerebellum (Kilts et al. 2001) while in heroin users cerebellar activitation has been correlated with self-reports of 'feeling tense' and 'withdrawal symptoms' during cue evoked craving (Sell et al. 2000). These findings support the idea that cerebellar activation may reflect aversive processing that is not specific to drug craving per se. In this respect, it is important to notice that the cerebellum is interconnected with the dopaminergic systems in the basal ganglia (Bostan et al. 2010) and even though this system is usually associated with positive reward (Drevets et al. 2001; Schultz 2007b; Leknes and Tracey 2008), there is evidence that dopamine neurons also respond to aversive events (Ungless, Magill, and Bolam 2004; Schultz 2007a; Leknes and Tracey 2008).

It has been proposed that cerebellum's principal function is '*modulation*', which would affect all the different domains based on the afferent functional input that the cerebellum receives, (Schmahmann 2004; D'Angelo and Casali 2012). "This theory suggests that the cerebellum could optimize performance by modulating behavior according to context, acting as an oscillation dampener. For example, the cerebellum may modulate emotional processes by integrating positive and negative affective inputs in the same way that it modulates fine motor control by integrating sensory inputs" (Moulton et al. 2014, page 319). The functional correlation between the cerebellum and the cortico-limbic

networks subserving emotion processing, executive control, drug craving, interoception and salience and its function as multimodal modulator indicates that the cerebellum plays a crucial role in maintaining the homeostatic balance between internal and external environment.

### 4.3. Direction of BOLD signal changes

In the present study we found a significant interaction between drug and setting in the region of the medial PFCx, in the striatum and the cerebellum. But interestingly, these regions shown an increased BOLD activity for the '*less preferred*' conditions such as "heroin outside the home" and "cocaine at home" compared to the '*preferred*' (heroin at home and cocaine outside the home'). Although the relationship between the BOLD signal and its underlying neural events is still not clear (Logothetis 2008), a possible interpretation is that a fronto-striatal-cerebellar network (see Figure 5) is responsible for the drug and setting interactions, and the specific increased activity may be related to the potential mismatch produced by the peripheral effects of the drugs and the external environment (see Chapter 2 for a full discussion of this issue). Thus, the sympathomimetic effects of cocaine may be encoded as potentially 'aversive' events if experienced in the home environment, compared to a non-home environment. The same line of reasoning may be applied to heroin, with the parasympatomimetic effects of heroin may be valued as 'aversive' in a non-domestic environments compared to a safer home setting as proposed by Badiani (2013).

### 4.4. Conclusions

The finding reported here demonstrate that setting of drug use exerts a substance-specific influence not only on the behavioural and subjective response to heroin and cocaine but also on the activity of brain regions implicated in processing drug reward and related information. The unforeseen involvement of the cerebellum in processing drug-setting interactions is in agreement with the recent theoretical model of cerebellar function (Schmahmann 2004; D'Angelo and Casali 2012) suggesting that this brain region modulates emotional processes by integrating positive and negative affective (Moulton et al. 2014).

Table 1. Socio-demographic information, diagnostic characteristics and pre-scan data of the study sample (*n*=20)

| | Mean (SD) |
|---|---|
| Age (years) | 35.35 (8.13) |
| Education (years) | 13.60 (3.31) |
| Employed | 85% |
| Handedness (left/ambidextrous/right) | 2/0/18 |
| Methadone program<br>mg/day | 95%<br>39.47 (30.00) |
| **Heroin** | |
| Years of use | 13.20 (6.29) |
| Age of first use[§] | 20.39 (4.30) |
| Main route of administration | |
| • Intravenous injection | 55% |
| • inhalation (smoked) | 35% |
| • insufflation (snorted) | 10% |
| **Cocaine** | |
| Years of use | 15.25 (5.73) |
| Age of first use[§] | 18.83 (6.86) |
| Main route of administration | |
| • Intravenous injection | 35% |
| • inhalation (smoked) | 10% |
| • insufflation (snorted) | 55% |
| **Pre-scan drug screen (% positive)[#]** | |
| • Methadone | 90% |
| • Morphine | 65% |
| • Cannabis | 55% |
| • Benzodiazepines | 20% |
| • Cocaine | 20% |
| • Barbiturates | 0% |
| • Amphetamine | 0% |
| • Methamphetamine | 0% |
| **Training Imagery Questionnaires**<br>'*Medium-High'* performance (<50% of the maximum score) | |
| • VVIQ | 90% |
| • VMIQ | 90% |
| • TVIC | 95% |
| • IIES–Positive emotional states | 90% |
| • IIES–Negative emotional states | 90% |

VVIQ, Vividness of Visual Imagery Questionnaire; VMIQ, Vividness of Movements Imagery Questionnaire; TVIC, Test of Visual Imagery Control; IIES; Imagery Induction of Emotional States (adapted version).

[§] Missing data for 2 participants

[#] Missing data for 1 participant

Table 2. Imagery scripts

| BASELINE IMAGERY AT HOME | BASELINE IMAGERY OUTSIDE THE HOME |
|---|---|
| 1) Imagine as vividly as possible that you are at home.<br>2) Imagine you are relaxing at home. | 1) Imagine as vividly as possible that you are in a club.<br>2) Imagine you are relaxing in the club. |
| **DRUG IMAGERY AT HOME** | **DRUG IMAGERY OUTSIDE THE HOME** |
| 1) Imagine as vividly as possible that you are at home.<br>2) Imagine to take heroin at home.<br><br>or<br><br>1) Imagine as vividly as possible that you are at home.<br>2) Imagine to take cocaine. | 1) Imagine as vividly as possible that you are in a club.<br>2) Imagine to take heroin in the club.<br><br>or<br><br>1) Imagine as vividly as possible that you are in a club.<br>2) Imagine to take cocaine in the club. |

Table 3. – Functional Regions of Interest (ROIs)

| *Hemisphere* | *ROI (probable Brodmann Area)* | *x* | *y* | *z* | *Dim (voxels)* | *Volume ($mm^3$)* |
|---|---|---|---|---|---|---|
| Left | Middle Frontal gyrus (BA44) | -39 | 11 | 34 | 69 | 1863 |
| Left | Inferior Frontal gyrus (BA45) | -51 | 26 | 25 | 88 | 2376 |
| Left | Inferior Parietal lobe (BA40 | -36 | -58 | 40 | 170 | 4590 |
| Left | Angular gyrus | -57 | -46 | 28 | 19 | 513 |
| Left | Inferior Frontal gyrus (BA45) | -48 | 44 | 7 | 17 | 459 |
| Left | Middle Frontal gyrus (BA46) | -33 | 56 | 19 | 4 | 108 |
| Left | Precentral gyrus (BA6) | -36 | 2 | 61 | 14 | 378 |
| Left | Middle Frontal gyrus (BA8) | -24 | 17 | 64 | 7 | 189 |
| Left | Caudate | -18 | 5 | 19 | 62 | 1674 |
| Left | Cerebellum (Crus I and Crus II) | -36 | -61 | -32 | 118 | 3186 |
| Left | Posterior Cingulum | -3 | -34 | 31 | 24 | 648 |
| Left | Precuneus (BA7) | -9 | -67 | 43 | 19 | 513 |
| Left | Supplementary motor area (BA32) | -6 | 20 | 46 | 8 | 216 |
| Left | Temporal gyrus (BA21) | -63 | -43 | -5 | 28 | 756 |
| Left | Temporal gyrus (BA21) | -60 | -13 | -11 | 70 | 1890 |
| Left | Thalamus | -3 | -10 | 10 | 64 | 1728 |
| Left | Brain stem | -3 | -28 | -14 | 11 | 297 |
| Right | Caudate | 18 | 5 | 16 | 38 | 1026 |
| Right | Cerebellum | 15 | -79 | -35 | 224 | 6048 |
| Right | Temporal gyrus (BA21) | 51 | -25 | -8 | 194 | 5238 |
| Right | Thalamus | 3 | -7 | 7 | 52 | 1404 |
| Right | Brain stem | 15 | -25 | -11 | 7 | 189 |

**Figure 1. Experimental Design and Vividness Ratings. A.** Overview of the imagery task. There were 8 trials, 2 for each combination of drug and setting. Each trial began with a baseline imagery period of 60 s, during which the participant were asked to imagine relaxing either at home or outside the home. The participants were then asked to imagine to use heroin or cocaine at home or in a club for 120 s (drug imagery). This period was followed by 60 s of rest, during which the participants were asked to not engage in imagery. Finally, the participants were asked to rate the vividness of the imagery on a VAS (1-10 points), by pressing a button. Immediately after completing the VAS the next trial began. The graph in panel B depicts the vividness rating for each participant after the imagery period (indicating no significant differences among the four conditions).

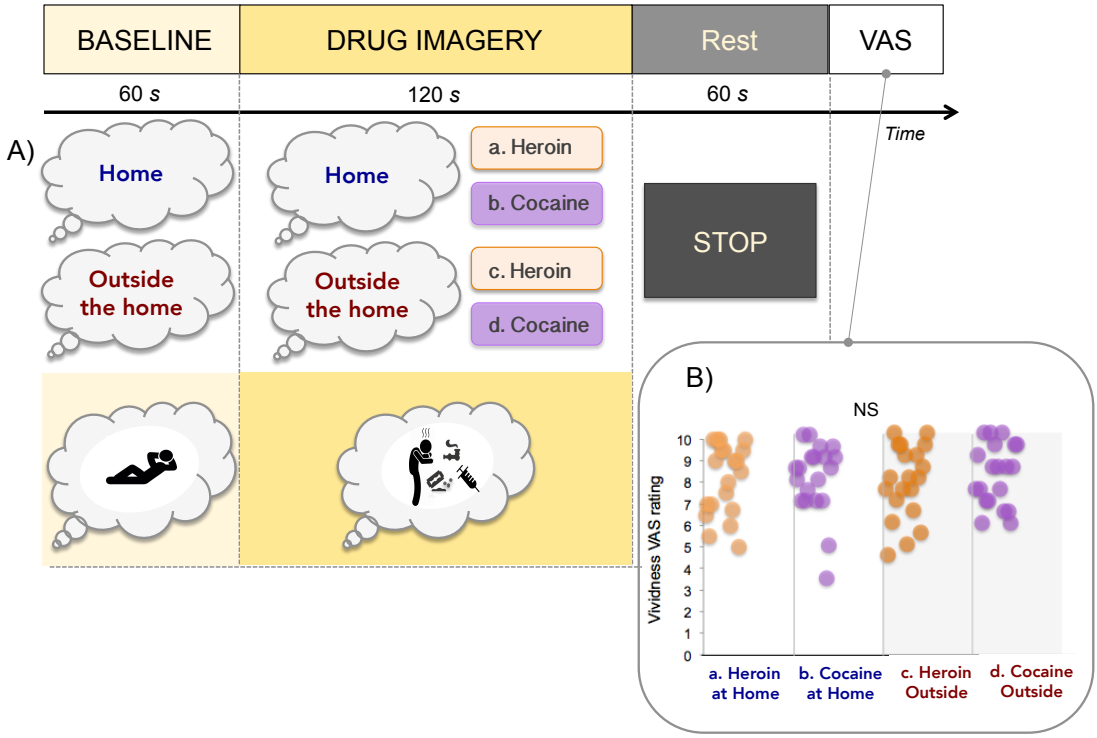


**Figure 2: Whole brain analysis map.** Neuronal activation during the drug imagery period was modeled as a box-car function spanning the whole duration of the imagery period and convolved with the hemodynamic response function (HRF) relative to the baseline imagery period. Whole brain analysis conducted in SPM8 using an "omnibus" F contrast, revealed significant changes in the regions showed below ($P_{FWE}$ <0.05, corrected for multiple comparison with family-wise error (FWE) at cluster level).

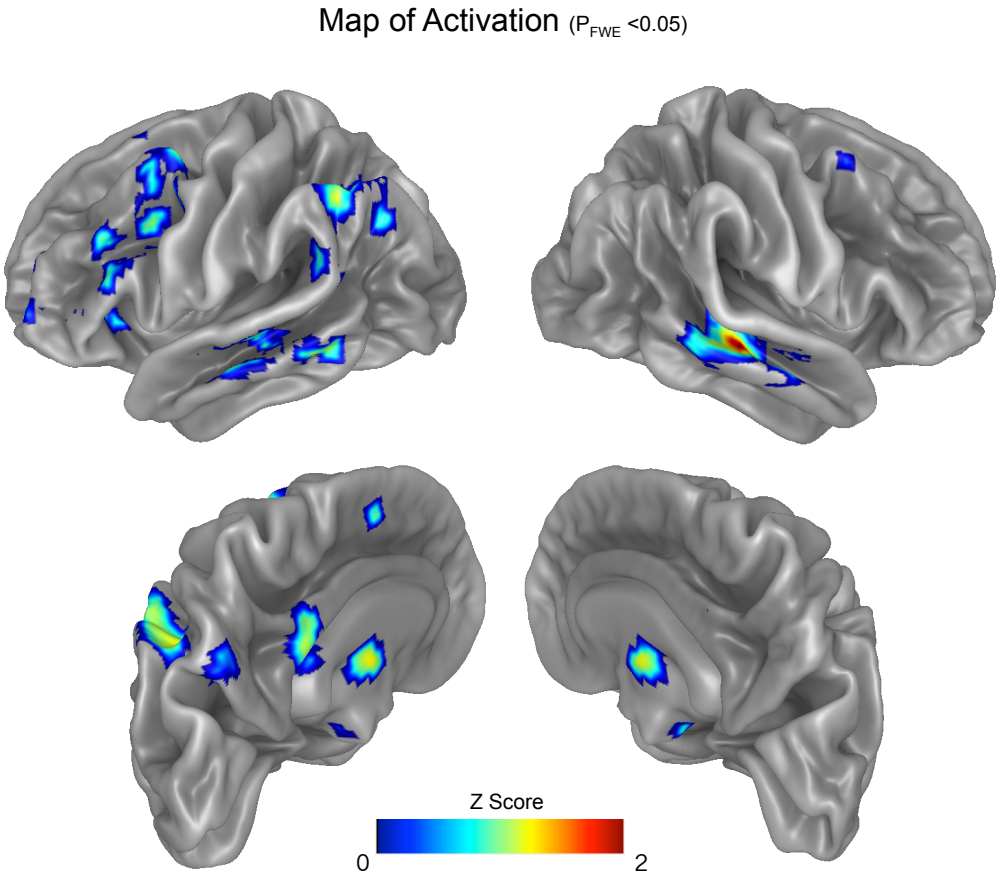


**Figure 3. Left PFCx.** Histograms (top panels) represent the mean (±SEM) change in BOLD signal during the drug imagery task (relative to baseline imagery) in the BA 46, BA 44, and BA 8 obtained by averaging the values of 12 10-s bins (FIR analysis, bottom panels).

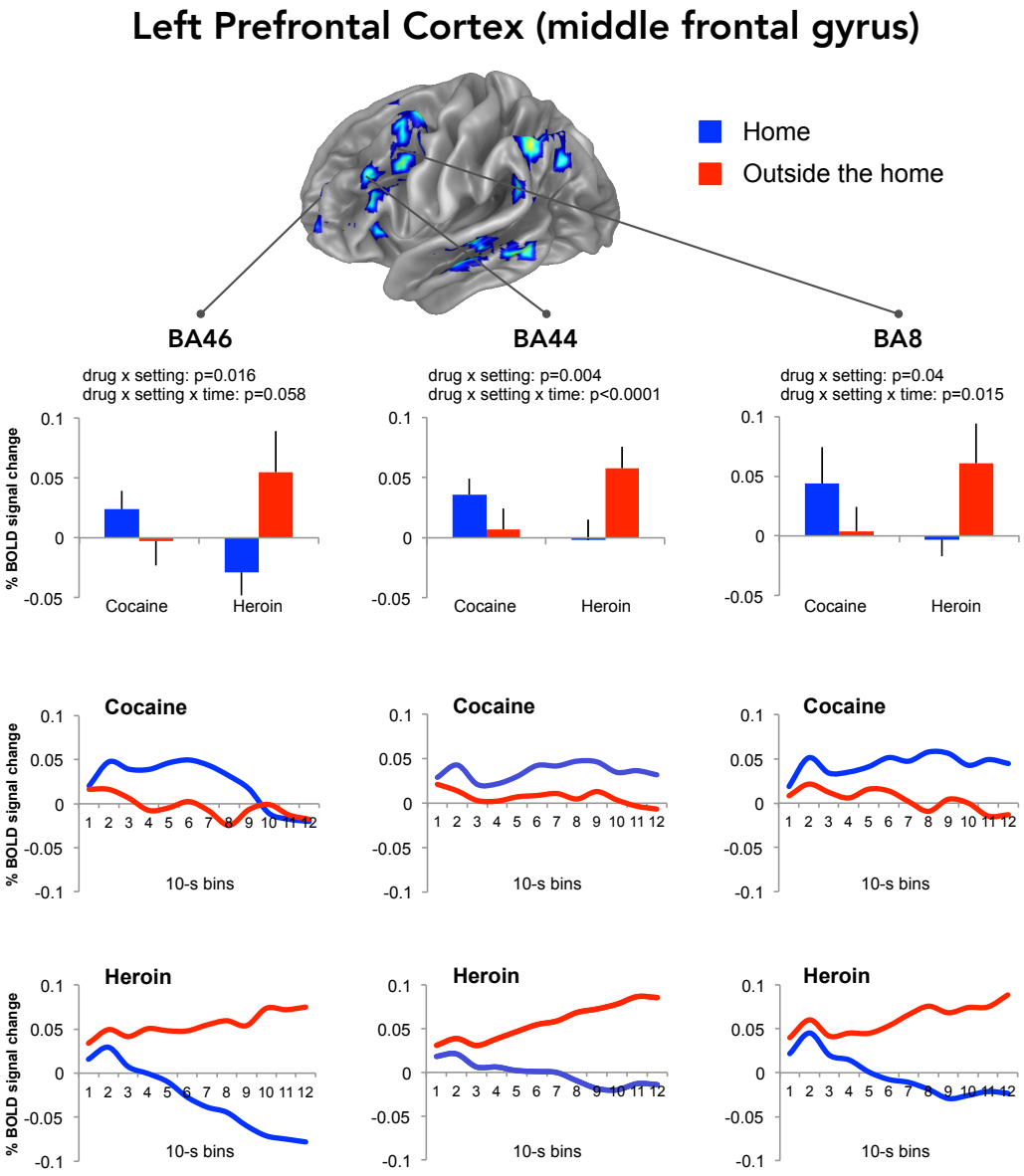


**Figure 4. Left caudate and cerebellum:** Histograms (top panels) represent the mean (±SEM) change in BOLD signal during the drug imagery task (relative to baseline imagery) in the left caudate obtained by averaging the values of 12 10-s bins (FIR analysis, bottom panels).

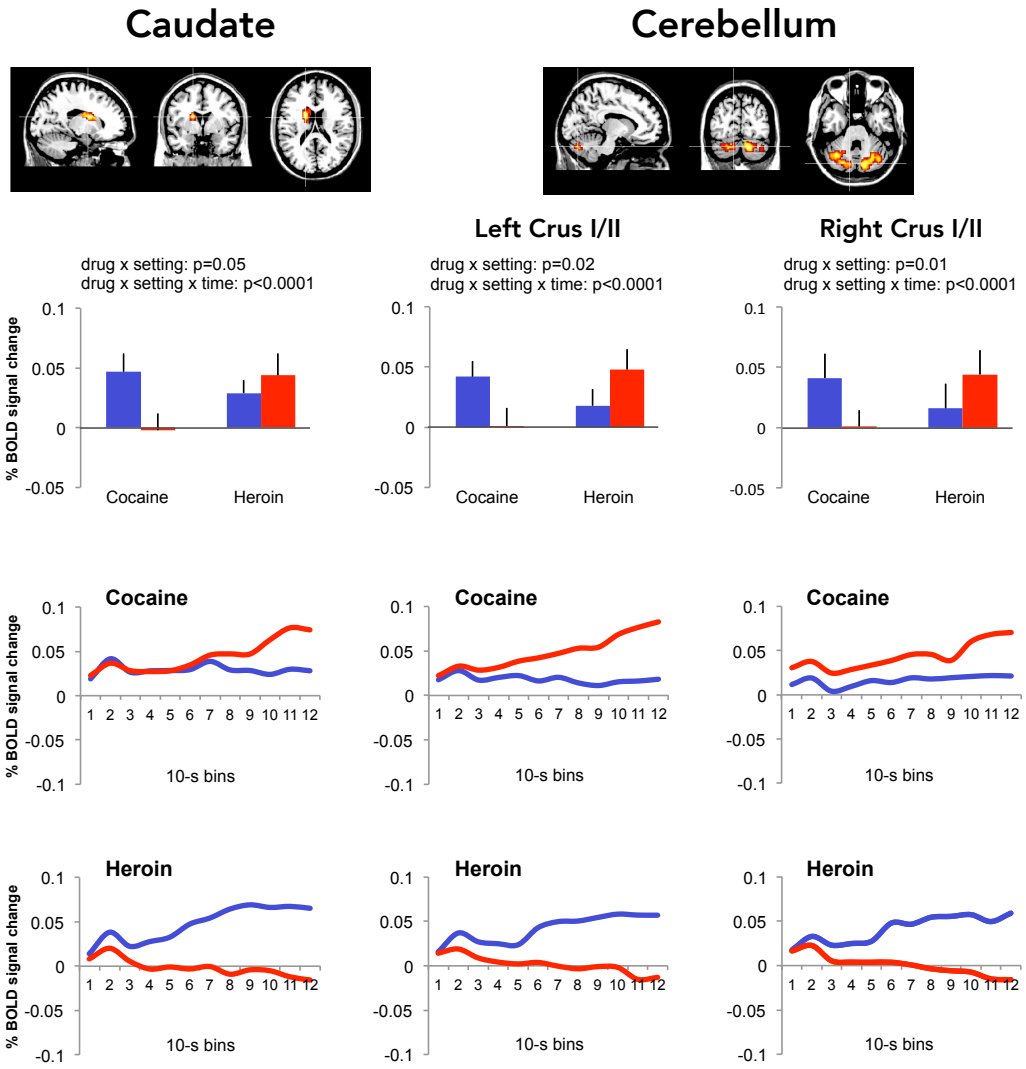


Figure 5. Fronto-striatal-cerebellar network.

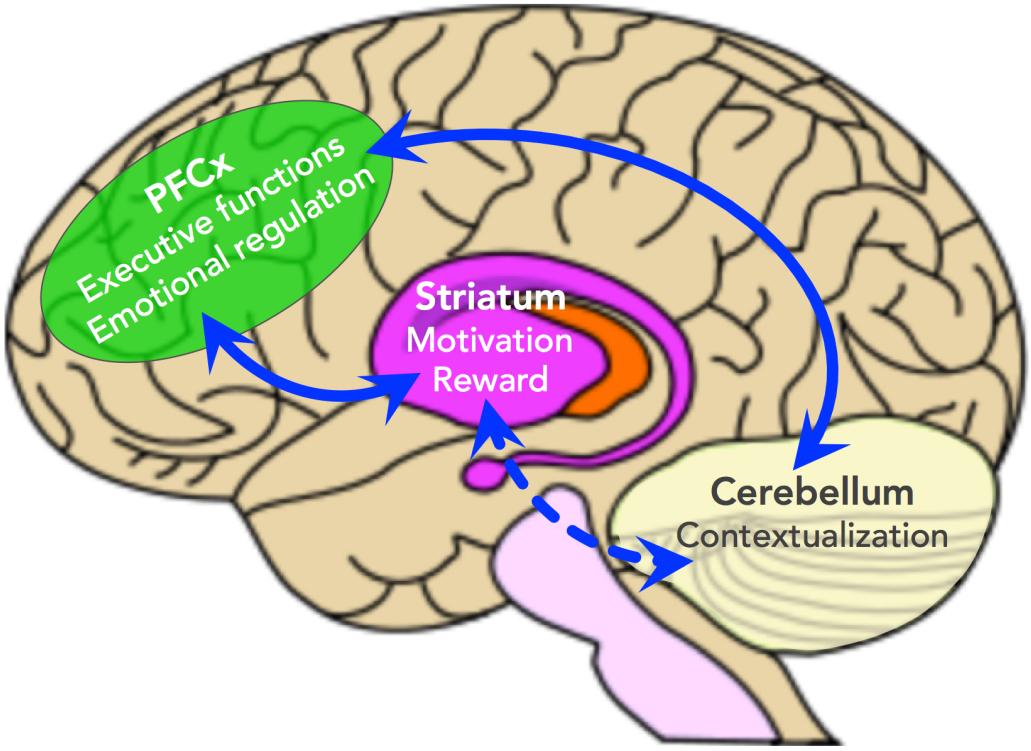
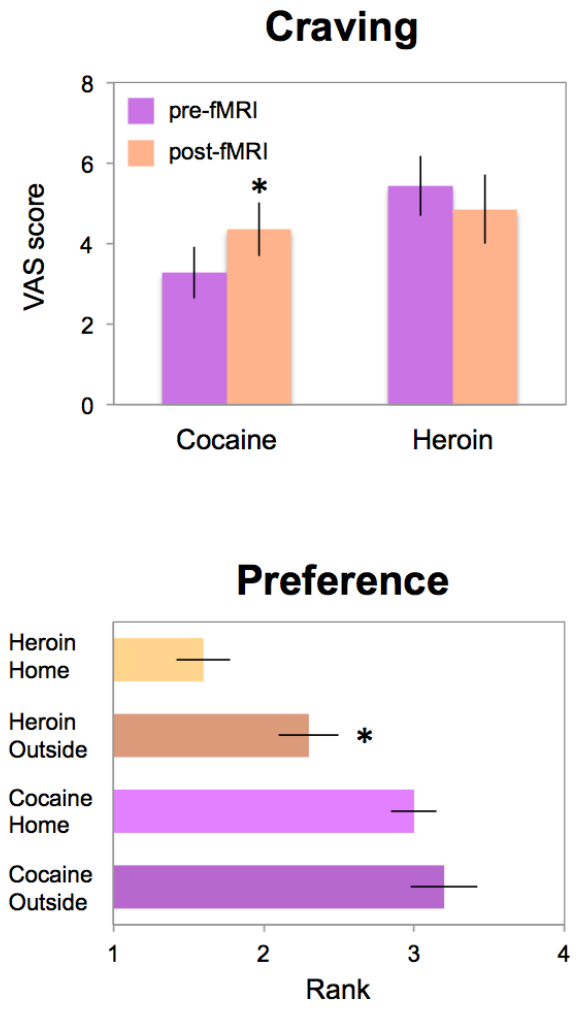


**Figure 6. Subjective Ratings of Drug Craving and Preference.**
**Drug Craving.** Mean (±SEM) craving (VAS scale) for heroin or cocaine before and after fMRI. * Cocaine pre- versus cocaine post-fMRI p=0.05. **Preference.** Mean (±SEM) ranking preference for the four combinations of drug and setting, ranging from 1 (most pleasurable) to 4 (least pleasurable). * Heroin at home versus heroin outside the home p=0.05.



## 5. References


Adamaszek, M., F. D'Agata, R. Ferrucci, C. Habas, S. Keulen, K. C. Kirkby, M. Leggio, et al. 2017. "Consensus Paper: Cerebellum and Emotion." *Cerebellum* 16 (2): 552–76.

Adamaszek, M., F. D'Agata, K. C. Kirkby, M. U. Trenner, B. Sehm, C. J. Steele, J. Berneiser, and K. Strecker. 2014. "Impairment of Emotional Facial Expression and Prosody Discrimination due to Ischemic Cerebellar Lesions." *Cerebellum* 13 (3): 338–45.

Avvisati, Riccardo, Laura Contu, Emiliana Stendardo, Caterina Michetti, Christian Montanari, Maria Luisa Scattoni, and Aldo Badiani. 2016. "Ultrasonic Vocalization in Rats Self-Administering Heroin and Cocaine in Different Settings: Evidence of Substance-Specific Interactions between Drug and Setting." *Psychopharmacology* 233 (8): 1501–11.

Badiani, Aldo. 2013. "Substance-Specific Environmental Influences on Drug Use and Drug Preference in Animals and Humans." *Current Opinion in Neurobiology* 23 (4): 588–96.

Badiani, Aldo, and Primavera A. Spagnolo. 2013. "Role of Environmental Factors in Cocaine Addiction." *Current Pharmaceutical Design* 19 (40): 6996–7008.

Badiani, A., M. M. Oates, H. E. Day, S. J. Watson, H. Akil, and T. E. Robinson. 1998. "Amphetamine-Induced Behavior, Dopamine Release, and c-Fos mRNA Expression: Modulation by Environmental Novelty." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 18 (24): 10579–93.

Badiani, A; Oates, M M; Day, H E; Watson, S J; Akil, H; Robinson, T E. 1999. "Environmental Modulation of Amphetamine-Induced c-Fos Expression in D1 versus D2 Striatal Neurons." *Behavioural Brain Research* 103 (2): 203–9.

Baumann, Oliver, and Jason B. Mattingley. 2012. "Functional Topography of Primary Emotion Processing in the Human Cerebellum." *NeuroImage* 61 (4): 805–11.

Behrmann, M., M. Moscovitch, and G. Winocur. 1994. "Intact Visual Imagery and Impaired Visual Perception in a Patient with Visual Agnosia." *Journal of Experimental Psychology. Human Perception and Performance* 20 (5): 1068–87.

Behrmann, M., G. Winocur, and M. Moscovitch. 1992. "Dissociation between Mental Imagery and Object Recognition in a Brain-Damaged Patient." *Nature* 359 (6396): 636–37.

Berger, Christopher C., and H. Henrik Ehrsson. 2014. "The Fusion of Mental Imagery and Sensation in the Temporal Association Cortex." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 34 (41): 13684–92.

Boileau, Isabelle, Alain Dagher, Marco Leyton, Krzysztof Welfeld, Linda Booij, Mirko Diksic, and Chawki Benkelfat. 2007. "Conditioned Dopamine Release in Humans: A Positron Emission Tomography [11C] Raclopride Study with Amphetamine." Journal of Neuroscience 27 (15). Soc Neuroscience: 3998-4003.

Bostan, Andreea C., Richard P. Dum, and Peter L. Strick. 2010. "The Basal Ganglia Communicate with the Cerebellum." *Proceedings of the National Academy of Sciences of the United States of America* 107 (18): 8452–56.

Breiter, H. C., R. L. Gollub, R. M. Weisskoff, D. N. Kennedy, N. Makris, J. D. Berke, J. M. Goodman, et al. 1997. "Acute Effects of Cocaine on Human Brain Activity and Emotion." *Neuron* 19 (3): 591–611.

Buckner, Randy L. 2013. "The Cerebellum and Cognitive Function: 25 Years of Insight from Anatomy and Neuroimaging." *Neuron* 80 (3): 807–15.

Buckner, Randy L., Fenna M. Krienen, Angela Castellanos, Julio C. Diaz, and B. T. Thomas Yeo. 2011. "The Organization of the Human Cerebellum Estimated by Intrinsic Functional Connectivity." *Journal of Neurophysiology* 106 (5): 2322–45.

Burock, Marc A., and Anders M. Dale. 2000. "Estimation and Detection of Event-Related fMRI Signals with Temporally Correlated Noise: A Statistically Efficient and Unbiased Approach." *Human Brain Mapping* 11 (4). Wiley Online Library: 249–60.

Caprioli, Daniele, Michele Celentano, Alessandro Dubla, Federica Lucantonio, Paolo Nencini, and Aldo Badiani. 2009. "Ambience and Drug Choice: Cocaine- and Heroin-Taking as a Function of Environmental Context in Humans and Rats." *Biological Psychiatry* 65 (10): 893–99.

Caprioli, Daniele, Michele Celentano, Giovanna Paolone, and Aldo Badiani. 2007b. "Modeling the Role of Environment in Addiction." *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 31 (8): 1639–53.

Caprioli, Daniele, Michele Celentano, Giovanna Paolone, Federica Lucantonio, Andrea Bari, Paolo Nencini, and Aldo Badiani. 2008. "Opposite Environmental Regulation of Heroin and Amphetamine Self-Administration in the Rat." *Psychopharmacology* 198 (3): 395–404.

Caprioli, Daniele, Giovanna Paolone, Michele Celentano, Arianna Testa, Paolo Nencini, and Aldo Badiani. 2007a. "Environmental Modulation of Cocaine Self-Administration in the Rat." *Psychopharmacology* 192 (3): 397–406.

Celentano, Michele, Daniele Caprioli, Pasqua Dipasquale, Pasqua Di Pasquale, Veronica Cardillo, Paolo Nencini, Silvana Gaetani, and Aldo Badiani. 2009. "Drug Context Differently Regulates Cocaine versus Heroin Self-Administration and Cocaine- versus Heroin-Induced Fos mRNA Expression in the Rat." *Psychopharmacology* 204 (2): 349–60.

Cichy, Radoslaw M., Jakob Heinzle, and John-Dylan Haynes. 2012. "Imagery and Perception Share Cortical Representations of Content and Location." *Cerebral Cortex* 22 (2): 372–80.

Costa, Vincent D., Peter J. Lang, Dean Sabatinelli, Francesco Versace, and Margaret M. Bradley. 2010. "Emotional Imagery: Assessing Pleasure and Arousal in the Brain's Reward Circuitry." *Human Brain Mapping* 31 (9): 1446–57.

Cox, Sylvia M. L., Chawki Benkelfat, Alain Dagher, J. Scott Delaney, France Durand, Samuel A. McKenzie, Theodore Kolivakis, Kevin F. Casey, and Marco Leyton. 2009. "Striatal Dopamine Responses to Intranasal Cocaine Self-Administration in Humans." *Biological Psychiatry* 65 (10): 846–50.

Cox, Sylvia M.L., Yvonne Yau, Kevin Larcher, France Durand, Theodore Kolivakis, J. Scott Delaney, Alain Dagher, Chawki Benkelfat, and Marco Leyton. 2017. "Cocaine Cue-Induced Dopamine Release in Recreational Cocaine Users." *Scientific Reports* 7 (April): 46665.

Craig, Arthur D. 2002. "Opinion: How Do You Feel? Interoception: The Sense of the Physiological Condition of the Body." *Nature Reviews. Neuroscience* 3 (8). Nature Publishing Group: 655.

Cuthbert, Bruce N., Peter J. Lang, Cyd Strauss, David Drobes, Christopher J. Patrick, and Margaret M. Bradley. 2003. "The Psychophysiology of Anxiety Disorder: Fear Memory Imagery." *Psychophysiology* 40 (3): 407–22.

D'Angelo, Egidio, and Stefano Casali. 2012. "Seeking a Unified Framework for Cerebellar Function and Dysfunction: From Circuit Operations to Cognition." *Frontiers in Neural Circuits* 6: 116.

Diano, M., F. D'Agata, F. Cauda, T. Costa, E. Geda, K. Sacco, S. Duca, D. M. Torta, and G. C. Geminiani. 2016. "Cerebellar Clustering and Functional Connectivity During Pain Processing." *Cerebellum* 15 (3): 343–56.

Dobromyslin, Vitaly I., David H. Salat, Catherine B. Fortier, Elizabeth C. Leritz, Christian F. Beckmann, William P. Milberg, and Regina E. McGlinchey. 2012. "Distinct Functional Networks within the Cerebellum and Their Relation to Cortical Systems Assessed with Independent Component Analysis." *NeuroImage* 60 (4): 2073–85.

Dougherty, D. D., L. M. Shin, N. M. Alpert, R. K. Pitman, S. P. Orr, M. Lasko, M. L. Macklin, A. J. Fischman, and S. L. Rauch. 1999. "Anger in Healthy Men: A PET Study Using Script-Driven Imagery." *Biological Psychiatry* 46 (4): 466–72.

Drevets, W. C., C. Gautier, J. C. Price, D. J. Kupfer, P. E. Kinahan, A. A. Grace, J. L. Price, and C. A. Mathis. 2001. "Amphetamine-Induced Dopamine Release in Human Ventral Striatum Correlates with Euphoria." *Biological Psychiatry* 49 (2): 81–96.

Everitt, Barry J., and Trevor W. Robbins. 2016. "Drug Addiction: Updating Actions to Habits to Compulsions Ten Years On." *Annual Review of Psychology* 67: 23–50.

Ferguson, Susan M., and Terry E. Robinson. 2004. "Amphetamine-Evoked Gene Expression in Striatopallidal Neurons: Regulation by Corticostriatal Afferents and the ERK/MAPK Signaling Cascade." *Journal of Neurochemistry* 91 (2). Wiley Online Library: 337–48.

Ferrucci, Roberta, Gaia Giannicola, Manuela Rosa, Manuela Fumagalli, Paulo Sergio Boggio, Mark Hallett, Stefano Zago, and Alberto Priori. 2012. "Cerebellum and Processing of Negative Facial Emotions: Cerebellar Transcranial DC Stimulation Specifically Enhances the Emotional Recognition of Facial Anger and Sadness." *Cognition & Emotion* 26 (5): 786–99.

Friston, K. J., P. Fletcher, O. Josephs, A. Holmes, M. D. Rugg, and R. Turner. 1998. "Event-Related fMRI: Characterizing Differential Responses." *NeuroImage* 7 (1): 30–40.

Ganis, Giorgio, William L. Thompson, and Stephen M. Kosslyn. 2004. "Brain Areas Underlying Visual Mental Imagery and Visual Perception: An fMRI Study." *Brain Research. Cognitive Brain Research* 20 (2): 226–41.

Goldstein, Rita Z., and Nora D. Volkow. 2002. "Drug Addiction and Its Underlying Neurobiological Basis: Neuroimaging Evidence for the Involvement of the Frontal Cortex." *The American Journal of Psychiatry* 159 (10): 1642–52.

Goldstein, Rita Z; Volkow, Nora D 2011. "Dysfunction of the Prefrontal Cortex in Addiction: Neuroimaging Findings and Clinical Implications." *Nature Reviews. Neuroscience* 12 (11): 652–69.

Gordon, Rosemary. 1949. "An Investigation Into Some Of The Factors That Favour The Formation Of Stereotyped Images." *The British Journal of Psychology. General Section* 39 (3). Blackwell Publishing Ltd: 156–67.

Habas, Christophe, Nirav Kamdar, Daniel Nguyen, Katherine Prater, Christian F. Beckmann, Vinod Menon, and Michael D. Greicius. 2009. "Distinct Cerebellar Contributions to Intrinsic Connectivity Networks." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 29 (26): 8586–94.

Hope, Bruce T., Danielle E. Simmons, Tim B. Mitchell, Justin D. Kreuter, and Brandi J. Mattson. 2006. "Cocaine-Induced Locomotor Activity and Fos Expression in Nucleus Accumbens Are Sensitized for 6 Months after Repeated Cocaine Administration Outside the Home Cage." *The European Journal of Neuroscience* 24 (3). Wiley Online Library: 867–75.

Isaac, Anne, David F. Marks, and David G. Russell. 1986. "An Instrument for Assessing Imagery of Movement: The Vividness of Movement Imagery Questionnaire (VMIQ)." *Journal of Mental Imagery*. Brandon House. http://psycnet.apa.org/psycinfo/1988-12630-001.

Ishai, A., and D. Sagi. 1995. "Common Mechanisms of Visual Imagery and Perception." *Science* 268 (5218): 1772–74.

Ito, Masao. 2006. "Cerebellar Circuitry as a Neuronal Machine." *Progress in Neurobiology* 78 (3-5): 272–303.

Ivry, R. 1997. "Cerebellar Timing Systems." *International Review of Neurobiology* 41: 555–73.

Kelly, Roberta M., and Peter L. Strick. 2003. "Cerebellar Loops with Motor Cortex and Prefrontal Cortex of a Nonhuman Primate." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 23 (23): 8432–44.

Kilts, C. D., J. B. Schweitzer, C. K. Quinn, R. E. Gross, T. L. Faber, F. Muhammad, T. D. Ely, J. M. Hoffman, and K. P. Drexler. 2001. "Neural Activity Related to Drug Craving in Cocaine Addiction." *Archives of General Psychiatry* 58 (4): 334–41.

Kosslyn, S. M., G. Ganis, and W. L. Thompson. 2001. "Neural Foundations of Imagery." *Nature Reviews. Neuroscience* 2 (9): 635–42.

Kriegeskorte, Nikolaus, W. Kyle Simmons, Patrick S. F. Bellgowan, and Chris I. Baker. 2009. "Circular Analysis in Systems Neuroscience: The Dangers of Double Dipping." *Nature Neuroscience* 12 (5): 535–40.

Kufahl, Peter R., Zhu Li, Robert C. Risinger, Charles J. Rainey, Gaohong Wu, Alan S. Bloom, and Shi-Jiang Li. 2005. "Neural Responses to Acute Cocaine Administration in the Human Brain Detected by fMRI." *NeuroImage* 28 (4): 904–14.

Lang, Peter J. 1979. "A Bio-Informational Theory of Emotional Imagery." *Psychophysiology* 16 (6). Wiley Online Library: 495–512.

Lang, Peter J., Michael J. Kozak, Gregory A. Miller, Daniel N. Levin, and Alvin McLean. 1980. "Emotional Imagery: Conceptual Structure and Pattern of

Somato-Visceral Response." *Psychophysiology* 17 (2). Blackwell Publishing Ltd: 179–92.

Lee, Sue-Hyun, Dwight J. Kravitz, and Chris I. Baker. 2012. "Disentangling Visual Imagery and Perception of Real-World Objects." *NeuroImage* 59 (4): 4064–73.

Leknes, Siri, and Irene Tracey. 2008. "A Common Neurobiology for Pain and Pleasure." *Nature Reviews. Neuroscience* 9 (4): 314–20.

Leyton, Marco, and Paul Vezina. 2013. "Striatal Ups and Downs: Their Roles in Vulnerability to Addictions in Humans." *Neuroscience and Biobehavioral Reviews* 37 (9 Pt A): 1999–2014.

Liu, Tao, Jianjun Li, Shixiong Huang, Changqinq Li, Zhongyan Zhao, Guoqiang Wen, and Feng Chen. 2017. "Altered Resting-State Functional Activity in Isolated Pontine Infarction Patients with Pathological Laughing and Crying." *Oncotarget*, July. doi:10.18632/oncotarget.19307.

Logothetis, Nikos K. 2008. "What We Can Do and What We Cannot Do with fMRI." *Nature* 453 (7197). Nature Publishing Group: 869.

Lupo, Michela, Elio Troisi, Francesca R. Chiricozzi, Silvia Clausi, Marco Molinari, and Maria Leggio. 2015. "Inability to Process Negative Emotions in Cerebellar Damage: A Functional Transcranial Doppler Sonographic Study." *Cerebellum* 14 (6): 663–69.

Marks, D. F. 1989. "Construct Validity of the Vividness of Visual Imagery Questionnaire." *Perceptual and Motor Skills* 69 (2): 459–65.

McTeague, Lisa M., Peter J. Lang, Marie-Claude Laplante, Bruce N. Cuthbert, Cyd C. Strauss, and Margaret M. Bradley. 2009. "Fearful Imagery in Social Phobia: Generalization, Comorbidity, and Physiological Reactivity." *Biological Psychiatry* 65 (5): 374–82.

Miller, Gregory A., Daniel N. Levin, Michael J. Kozak, Edwin W. Cook, Alvin McLean, and Peter J. Lang. 1987. "Individual Differences in Imagery and the Psychophysiology of Emotion." *Cognition and Emotion* 1 (4). Routledge: 367–90.

Miquel, Marta, Rebeca Toledo, Luis I. Garcia, Genaro A. Coria-Avila, and Jorge Manzo. 2009. "Why Should We Keep the Cerebellum in Mind When Thinking About Addiction?" *Current Drug Abuse Reviews* 2 (1): 26–40.

Montanari, Christian, Emiliana Stendardo, Maria Teresa De Luca, Maria Meringolo, Laura Contu, and Aldo Badiani. 2015. "Differential Vulnerability to Relapse into Heroin versus Cocaine-Seeking as a Function of Setting." *Psychopharmacology* 232 (13): 2415–24.

Moulton, Eric A., Igor Elman, Lino R. Becerra, Rita Z. Goldstein, and David Borsook. 2014. "The Cerebellum and Addiction: Insights Gained from Neuroimaging Research." *Addiction Biology* 19 (3): 317–31.

Moulton, Eric A., Igor Elman, Gautam Pendse, Jeremy Schmahmann, Lino Becerra, and David Borsook. 2011. "Aversion-Related Circuitry in the Cerebellum: Responses to Noxious Heat and Unpleasant Images." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 31 (10): 3795–3804.

Ollinger, J. M., G. L. Shulman, and M. Corbetta. 2001. "Separating Processes within a Trial in Event-Related Functional MRI I. The Method." *NeuroImage* 13 (1): 210–17.

Owen, Adrian M., Martin R. Coleman, Melanie Boly, Matthew H. Davis, Steven Laureys, and John D. Pickard. 2006. "Detecting Awareness in the Vegetative State." *Science* 313 (5792): 1402.

Paolone, Giovanna, David Conversi, Daniele Caprioli, Paola Del Bianco, Paolo Nencini, Simona Cabib, and Aldo Badiani. 2007. "Modulatory Effect of Environmental Context and Drug History on Heroin-Induced Psychomotor Activity and Fos Protein Expression in the Rat Brain." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 32 (12): 2611–23.

Paolone, Giovanna, Margherita Palopoli, M. Cristina Marrone, Paolo Nencini, and Aldo Badiani. 2004. "Environmental Modulation of the Interoceptive Effects of Amphetamine in the Rat." *Behavioural Brain Research* 152 (1): 149–55.

Park, Ji-Young, Bon-Mi Gu, Do-Hyung Kang, Yong-Wook Shin, Chi-Hoon Choi, Jong-Min Lee, and Jun Soo Kwon. 2010. "Integration of Cross-Modal Emotional Information in the Human Brain: An fMRI Study." *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior* 46 (2): 161–69.

Paulus, Martin P., and Jennifer L. Stewart. 2014. "Interoception and Drug Addiction." *Neuropharmacology* 76 Pt B (January): 342–50.

Poldrack, Russell A. 2007. "Region of Interest Analysis for fMRI." *Social Cognitive and Affective Neuroscience* 2 (1): 67–70.

Reddy, Leila, Naotsugu Tsuchiya, and Thomas Serre. 2010. "Reading the Mind's Eye: Decoding Category Information during Mental Imagery." *NeuroImage* 50 (2): 818–25.

Risinger, Robert C., Betty Jo Salmeron, Thomas J. Ross, Shelley L. Amen, Michael Sanfilipo, Raymond G. Hoffmann, Alan S. Bloom, Hugh Garavan, and Elliot A. Stein. 2005. "Neural Correlates of High and Craving during Cocaine Self-Administration Using BOLD fMRI." *NeuroImage* 26 (4): 1097–1108.

Rollins, Mark. 1992. *The American Journal of Psychology* 105 (1). University of Illinois Press: 146–52.

Scheuerecker, J., T. Frodl, N. Koutsouleris, T. Zetzsche, M. Wiesmann, A. M. Kleemann, H. Brückmann, G. Schmitt, H-J Möller, and E. M. Meisenzahl. 2007. "Cerebral Differences in Explicit and Implicit Emotional Processing--an fMRI Study." *Neuropsychobiology* 56 (1): 32–39.

Schmahmann, J. D. 1991. "An Emerging Concept. The Cerebellar Contribution to Higher Function." *Archives of Neurology* 48 (11): 1178–87.

Schmahmann, J.D. 1996. "From Movement to Thought: Anatomic Substrates of the Cerebellar Contribution to Cognitive Processing." *Human Brain Mapping* 4 (3): 174–98.

Schmahmann, J. D., and J. C. Sherman. 1998. "The Cerebellar Cognitive Affective Syndrome." *Brain: A Journal of Neurology* 121 ( Pt 4) (April): 561–79.

Schmahmann, J. D., J. B. Weilburg, and J. C. Sherman. 2007. "The Neuropsychiatry of the Cerebellum—insights from the Clinic." *Cerebellum* . Springer. http://www.springerlink.com/index/L272611203113641.pdf.

Schmahmann, Jeremy D. 2004. "Disorders of the Cerebellum: Ataxia, Dysmetria of Thought, and the Cerebellar Cognitive Affective Syndrome." *The Journal of Neuropsychiatry and Clinical Neurosciences* 16 (3): 367–78.

Schultz, Wolfram. 2007a. "Multiple Dopamine Functions at Different Time Courses." *Annual Review of Neuroscience* 30: 259–88.

Schultz, Wolfram. 2007b. "Behavioral Dopamine Signals." *Trends in Neurosciences* 30 (5): 203–10.

Sell, L. A., J. S. Morris, J. Bearn, R. S. Frackowiak, K. J. Friston, and R. J. Dolan. 2000. "Neural Responses Associated with Cue Evoked Emotional States and Heroin in Opiate Addicts." *Drug and Alcohol Dependence* 60 (2): 207–16.

Shinn, Ann K., Youkyung S. Roh, Caitlin T. Ravichandran, Justin T. Baker, Dost Öngür, and Bruce M. Cohen. 2017. "Aberrant Cerebellar Connectivity in Bipolar Disorder with Psychosis." *Biological Psychiatry. Cognitive Neuroscience and Neuroimaging* 2 (5): 438–48.

Sinha, Rajita. 2009. "Modeling Stress and Drug Craving in the Laboratory: Implications for Addiction Treatment Development." *Addiction Biology* 14 (1): 84–98.

Steffener, Jason, Matthias Tabert, Aaron Reuben, and Yaakov Stern. 2010. "Investigating Hemodynamic Response Variability at the Group Level Using Basis Functions." *NeuroImage* 49 (3): 2113–22.

Stoodley, Catherine J. 2012. "The Cerebellum and Cognition: Evidence from Functional Imaging Studies." *Cerebellum* 11 (2): 352–65.

Talairach, J., and P. Tournoux. 1988. *Co-Planar Stereotaxic Atlas of the Human Brain. 3-Dimensional Proportional System: An Approach to Cerebral Imaging*. Thieme.

Turner, Beth M., Sergio Paradiso, Cherie L. Marvel, Ronald Pierson, Laura L. Boles Ponto, Richard D. Hichwa, and Robert G. Robinson. 2007. "The Cerebellum and Emotional Experience." *Neuropsychologia* 45 (6): 1331–41.

Ungless, Mark A., Peter J. Magill, and J. Paul Bolam. 2004. "Uniform Inhibition of Dopamine Neurons in the Ventral Tegmental Area by Aversive Stimuli." *Science* 303 (5666): 2040–42.

Van Overwalle, Frank, Kris Baetens, Peter Mariën, and Marie Vandekerckhove. 2015. "Cerebellar Areas Dedicated to Social Cognition? A Comparison of Meta-Analytic and Connectivity Results." *Social Neuroscience* 10 (4): 337–44.

Volkow, N. D., G. J. Wang, J. S. Fowler, M. Fischman, R. Foltin, N. N. Abumrad, S. J. Gatley, et al. 1999. "Methylphenidate and Cocaine Have a Similar in Vivo Potency to Block Dopamine Transporters in the Human Brain." *Life Sciences* 65 (1): PL7–12.

Volkow, Nora D., Gene-Jack Wang, Joanna S. Fowler, and Dardo Tomasi. 2012. "Addiction Circuitry in the Human Brain." *Annual Review of Pharmacology and Toxicology* 52: 321–36.

Volkow, Nora D., Gene-Jack Wang, Frank Telang, Joanna S. Fowler, Jean Logan, Anna-Rose Childress, Millard Jayne, Yeming Ma, and Christopher Wong. 2006. "Cocaine Cues and Dopamine in Dorsal Striatum: Mechanism of Craving in Cocaine Addiction." *The Journal of Neuroscience*: *The Official Journal of the Society for Neuroscience* 26 (24): 6583–88.

Watson, Ben J., Lindsay G. Taylor, Alastair G. Reid, Sue J. Wilson, Paul R. Stokes, David J. Brooks, James F. Myers, Federico E. Turkheimer, David J. Nutt, and Anne R. Lingford-Hughes. 2014. "Investigating Expectation and Reward in Human Opioid Addiction with [(11) C]raclopride PET." *Addiction Biology* 19 (6): 1032–40.

Wong, Dean F., Hiroto Kuwabara, David J. Schretlen, Katherine R. Bonson, Yun Zhou, Ayon Nandi, James R. Brasić, et al. 2006. "Increased Occupancy of Dopamine Receptors in Human Striatum during Cue-Elicited Cocaine Craving." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 31 (12): 2716–27.

Wright, Jack, and Walter Mischel. 1982. "Influence of Affect on Cognitive Social Learning Person Variables." *Journal of Personality and Social Psychology* 43 (5). American Psychological Association: 901.

Zinberg, Norman E. 1984. "Drug, Set and Setting." *New Haven: Yale University*.

# Chapter 4

## General Discussion

### 1. Aims of the dissertation

Previous studies in animals and humans have shown that the setting of drug taking can exert a substance-specific influence on the response to heroin and cocaine (for a review, see Chapter 1). On the basis of these findings, a novel hypothesis of drug reward was proposed to explain the role of setting in modulating drug reward (Badiani 2013). According to this hypothesis, it has been proposed (Badiani 2013) that the overall rewarding effects of addictive drugs are determined not only by their euphoriant effects or by their ability to activate the neural mechanism of incentive salience (Berridge et al. 2009) but by the emotional appraisal of central and peripheral drug effects as a function of the setting of drug use. Specifically, it was hypothesized that the interoceptive information produced by the central and peripheral effects of drugs is evaluated against the background of the exteroceptive information related to setting of drug use. In the presence of a *mismatch* between interoceptive and exteroceptive information, the positive valence of drug experience would be reduced. For example, the state of central and peripheral arousal (usually associated by exposure to danger) produced by psychostimulant drugs such as cocaine would generate, when cocaine is taken in a safe home environment, a mismatch between exteroceptive and interoceptive information. Likewise, the state of central and peripheral

sedation (usually experienced in safe, familiar environments) produced by heroin would result in a mismatch when heroin is taken in potentially unsafe settings. This hypothesis could be put to test only using a comparative approach, that is by studying the effects of cocaine and heroin side by side and possibly within-subject.

The first aim of this dissertation was to investigate the role of setting in modulating the affective state induced by heroin versus cocaine in real world addicts who co-abused both drugs (see Chapter 2). I first collected retrospective information on the subjective central and peripheral effects of heroin and cocaine and then assessed the affective state experienced while under the effect of these drugs using a novel test developed on the basis on the Circumplex Model of Affect (Russell 1980).

The second aim of my dissertation was to test a second prediction of the working hypothesis: the setting of drug use should modulate in opposite direction the effects of heroin and cocaine on brain reward regions. Thus, I conducted an emotional imagery study coupled with fMRI to investigate the interaction of drug setting on the brain activity of human co-abusers (see Chapter 3).

## 2. Main findings

The present dissertation reports four major findings.

## 2.1. Subjective appraisal of the central and peripheral effects of heroin and cocaine

Heroin and cocaine produced distinct constellations of central and peripheral effects. Most important, the spectra of effects of the two drugs were almost the opposite in mimicking the activity of the autonomic nervous system: cocaine producing a sympathomimetic pattern and heroin a parasympathomimetic-like pattern. Thus, opposite interoceptive information was generated by the actions of heroin and cocaine in the brain and body of the addicts. Given the role of the autonomic nervous in emotional processing (Kreibig 2010, Levenson 2014) it is reasonable to assume that this information contributed to a differential emotional appraisal of heroin versus cocaine described below.

To the best of my knowledge, this is the first study to directly compare the subjective effects of cocaine versus heroin in human addicts.

## 2.2. Shift in the emotional valence of heroin and cocaine as a function of setting

The affective state induced by heroin and cocaine changed as a function of the setting of drug use. As expected heroin produced, both in the home and the non home setting, a state of sedation in most addicts. However, in agreement with the 'mismatch' hypothesis highlighted above, most addicts reported that the overall experience was pleasurable when heroin was taken at home but not when taken outside the home. In contrast, cocaine produced (regardless of setting) a state of arousal in most addicts. Again in agreement with the 'mismatch' hypothesis, the overall experience resulted more pleasurable when cocaine was taken outside the home than when taken outside the home.

To the best of my knowledge, this is the first study to compare the emotional appraisal of cocaine versus heroin use in human addicts.

### 2.3. Drug 'pleasure'

A corollary of the finding described above is represented by the fact that it provides further evidence that addictive drugs do not always produce a pleasurable affective state. Actually, we found that in certain settings almost two-thirds of experienced drug users reported a mainly unpleasant affective state under the influence of cocaine. This confirms that addictive drugs can affect in a very different manner distinct aspects of the rewarding process as suggested by Berridge and colleagues (Smith et al. 2011; Berridge et al. 2009).

### 2.4. Differential patterns of brain activation as a function of drug and setting

Consistent with our working hypothesis, we found that cocaine- and heroin-related imagery in addicts induced distinct patterns of activity in the prefrontal cortex, caudate, and cerebellum brain activation and, most important, that the setting of drug taking influenced in opposite direction the changes in BOLD signal in these regions. Furthermore, we found quite surprisingly the same pattern of dissociation in the cerebellum, indicating the importance of further investigating the role of this structure in modulating positive and negative affect.

To the best of my knowledge, this is the first study to directly compare cocaine and heroin in an emotional imagery task coupled with fMRI.

## 3. General Discussion

For a full discussion of the results reported here see Chapter 2 and 3.

Overall, these studies clearly show that the affective and neurobiological states experienced when taking heroin and cocaine are not a simple function of drug actions. These findings support the hypothesis (Badiani 2013) that the affective state resulting from drug use is the result of an interaction between *exteroceptive* information (related to the setting) and *interoceptive* information (including the specific central and peripheral effects of the drug). On the basis of these earlier findings, we proposed that the affective valence of cocaine is thwarted when the drug is taken in a home environment because of the mismatch between exteroceptive information (i.e., safe home environment) and interoceptive information (i.e., the central and peripheral arousal produced by cocaine). Similarly, the affective valence of heroin is thwarted when the drug is taken in an exciting, potentially dangerous environment because of the mismatch between the latter and the state of sedation produced by heroin. The findings reported here indicate that this is indeed the case. Using a novel bi-dimensional test developed on the basis of the Circumplex Model of Affect (Russel 1980), we found that the affective state produced by heroin was indeed appraised as more pleasant when the drug was used at home then when it was when used outside the home, whereas the affective state produced by cocaine was more pleasant when the drug was used outside the home then when used at home. More specifically, our data confirmed that the shift in the affective valence of heroin

occurred in association with its sedative effects, whereas the shift in the affective valence of cocaine occurred in association with its arousing effects. It is reasonable to assume that the sympathomimetic effects of cocaine and the parasympathomimetic-like effect of heroin contributed to generate the emotional state of arousal and sedation produced by cocaine and heroin, respectively (Levenson 2014; Kreibig 2010). Indeed, we have found that under the influence of cocaine addicts perceive an increase in heart rate, respiratory rate, and muscular tension, as well as a decrease in salivation. In contrast, when the same individuals were under the influence of heroin, they perceived a reduction in heart rate and respiratory rate.

Our working hypothesis also predicted that the setting should alter in opposite directions the effects of heroin and cocaine in the PFCx and the striatum, regions implicated in brain reward in humans. Indeed, we found a significant interaction between drug and setting in the PFCx, in the striatum and the cerebellum. Interestingly, these regions showed an increased BOLD activity for the 'less preferred' conditions such as "heroin outside the home" and "cocaine at home" compared to the 'preferred' ones (heroin at home and cocaine outside the home'). Although the relationship between the BOLD signal and its underlying neural events is still not clear (Logothetis 2008), a possible interpretation is that a fronto-striatal-cerebellar network is responsible for the drug and setting interactions, and the specific increased activity may be related to the potential mismatch produced by the peripheral effects of the drugs and the external environment.

It is important to note here that the opposite spectra of action of heroin and cocaine on the autonomic nervous system and the differential role setting in modulating affective valence (see Chapter 2) may be somewhat related to

the unforeseen involvement of the Cerebellum in processing drug and setting interaction (see Chapter 3). Indeed, a recent Magneto-Encephalography (MEG) study revealed a differential spatiotemporal profile in the neural responses within the cerebellum during the processing of emotion along the arousal and valence dimensions, suggesting a complex role of the cerebellum in emotional processing (Styliadis et al. 2015). Furthermore, it has been shown that patients with cerebellar lesions exhibit a decrease in heart rate associated with fear conditioning and decreased skin conductance in response to negative stimuli (Maschke et al. 2002; Annoni et al. 2003). These studies provide support for a critical role for the cerebellum in integrating the autonomic responses and higher-order neural circuitry involved in emotional processing, with a particular role of the cerebellar involvement in the implicit responses mediated by the autonomic neural pathways that subserve the cardiovascular system and emotional regulation (Blood et al. 2015). A possible interpretation is that the cerebellum is a cerebral structure able to rapidly synchronize the motor, sensory and emotional processing to the complexity of the external and internal environment by serial and parallel computations networks involved in stimulus perception (Snow et al. 2014; Baumann et al. 2015).

Although it was not the major aim of the present study, the collection of data concerning the emotional valence of the drug experience gave us the opportunity to address an important issue in the field of drug addiction research. It is often thought even by experts that all addictive drugs "directly or indirectly target the brain's reward system by flooding the circuit with dopamine […] in regions of the brain that regulate […] feelings of pleasure. The overstimulation of this system, which rewards our natural behaviors,

produces the euphoric effects sought by people who use drugs and teaches them to repeat the behavior." (https://www.drugabuse.gov/publications/media-guide/science-drug-abuse-addiction-basics). Our data challenge this notion by showing that even prototypical addictive drugs like heroin and cocaine do not necessarily produce a pleasurable affective state in all contexts. Actually, in certain settings almost two-thirds of experienced drug users report that cocaine produces a mainly unpleasant affective state even during the period of regular use, although they still use the drug in such settings. This is consistent with the theory that the mechanisms underlying the motivation to use drugs are separable from those implicated in generating drug 'pleasure' (Robinson & Berridge 2008; Berridge & Kringelbach 2013).

In summary, the data reported here demonstrate that setting of drug use exerts a substance-specific influence not only on the behavioural and subjective response to heroin and cocaine but also on the activity of brain regions implicated in processing drug reward and related information.

The within-subject design of our studies makes the findings especially compelling, because the results cannot be easily ascribed to differences in drug availability, peer influence or other socio-demographic factors. These findings may help to explain epidemiological data indicating unique environmental influences on heroin versus cocaine abuse (Kendler et al. 2003) and suggest that therapeutic approaches to the treatment of drug addiction should take into account the distinctive effects of different classes of drugs as well as the contexts of drug use.

### 3.1. Limitations

The studies reported here present a number of limitations. In the first place, it relies on retrospective reports and subjective appraisal. As discussed in the next section, technological developments may provide more objective measures of drug effects in real world addicts without interfering with their daily life. However, for the time being these techniques are still in their infancy. Furthermore, as discussed in Chapter 2, the risk of conscious or unconscious biases in relaying information by drug addicts, and humans in general has been often emphasized. However, in our case, it is difficult to see in what way and for what reason our participants should have provided us with biased information, as they were not aware of the hypothesis being tested and the nature of our questionnaires and tasks was not likely to elicit answers that 'pleases' the researchers. Finally, the within-subject structure of our studies, with repeated measures for heroin and cocaine in the same individuals, further reduced the probability that individual biases contributed to the produce the dissociations observed.

Another potential limitation of my dissertation is represented by the lack of measures taken while the addicts are under the direct influence of drugs. We are currently conducting studies to add this type of information. However, it should be noted that there are ethical and procedural constraints that make this type of studies more difficult to conduct. Furthermore, the nature of the experiments that could be done under controlled conditions deviates substantially from the real-world characteristics of drug taking. Finally, it should be noted that the complex pharmacological actions of drugs

can complicate the interpretation of findings obtained with behavioural testing and imaging techniques.

### 3.2. Future directions

The hypothesis explored in the present dissertation could be further explored using methodologies that are still under development. For example, the increasing use of new technology for interventional purposes, such as smartphones and non-invasive sensor-based technologies had opened up new opportunities for monitoring behaviour and affective states of people in real-time in their daily environment (Kanjo et al. 2015). Affective computing is a recent area, which focuses on the development of systems able to detect, interpret and possibly deliver feedback "all the time everywhere" (Kanjo et al. 2015, page 1198). The increasing popularity of wearable and wireless devices might allow researchers to measure physiological signals (i.e., galvanic skin conductance, heart rate variability) over an extended period of time with minimal interference with the users' normal activity. The joint use of daily self-report and physiological measures not only can help to obtain richer information and to formulate finer theoretical interpretations. Indeed, it is hoped that by using Ecological Momentary Assessment (EMA) methodology it will be possible to gather data not obtainable in controlled experimental settings (Stone and Shiffman 1994; Epstein et al. 2009; Horvath et al. 2017). Furthermore, the use of EMA can have a therapeutic application and support the rehabilitation process. Indeed, the ability to gather information about the patients' physiological and emotional state, to assess their symptomatology, and detect possible risk factors, seems to offer a valuable resource in support

of behavioural change therapy, providing tools for instant and tailored clinical interventions.

## 6. References


Annoni, Jean-Marie, Radek Ptak, Anne-Sarah Caldara-Schnetzer, Asaid Khateb, and Branka Zei Pollermann. 2003. "Decoupling of Autonomic and Cognitive Emotional Reactions after Cerebellar Stroke." *Annals of Neurology* 53 (5): 654–58.

Badiani, Aldo. 2013. "Substance-Specific Environmental Influences on Drug Use and Drug Preference in Animals and Humans." *Current Opinion in Neurobiology* 23 (4): 588–96.

Baumann, Oliver, Ronald J. Borra, James M. Bower, Kathleen E. Cullen, Christophe Habas, Richard B. Ivry, Maria Leggio, et al. 2015. "Consensus Paper: The Role of the Cerebellum in Perceptual Processes." *Cerebellum* 14 (2). Springer US: 197–220.

Berridge, Kent C., and Morten L. Kringelbach. 2013. "Neuroscience of Affect: Brain Mechanisms of Pleasure and Displeasure." *Current Opinion in Neurobiology* 23 (3): 294–303.

Berridge, Kent C., Terry E. Robinson, and J. Wayne Aldridge. 2009. "Dissecting Components of Reward: 'Liking', 'Wanting', and Learning." *Current Opinion in Pharmacology* 9 (1): 65–73.

Blood, Julia D., Jia Wu, Tara M. Chaplin, Rebecca Hommer, Lauren Vazquez, Helena J. V. Rutherford, Linda C. Mayes, and Michael J. Crowley. 2015. "The Variable Heart: High Frequency and Very Low Frequency Correlates of Depressive Symptoms in Children and Adolescents." *Journal of Affective Disorders* 186 (November): 119–26.

Epstein, David H., Jessica Willner-Reid, Massoud Vahabzadeh, Mustapha Mezghanni, Jia-Ling Lin, and Kenzie L. Preston. 2009. "Real-Time Electronic Diary Reports of Cue Exposure and Mood in the Hours before Cocaine and Heroin Craving and Use." *Archives of General Psychiatry* 66 (1): 88–94.

Horvath, Keith J., Sara Lammert, Sara LeGrand, Kathryn E. Muessig, and José A. Bauermeister. 2017. "Using Technology to Assess and Intervene with Illicit Drug-Using Persons at Risk for HIV." *Current Opinion in HIV and AIDS* 12 (5): 458–66.

Kanjo, Eiman, Luluah Al-Husain, and Alan Chamberlain. 2015. "Emotions in Context: Examining Pervasive Affective Sensing Systems, Applications, and Analyses." *Personal and Ubiquitous Computing* 19 (7). Springer London: 1197–1212.

Kendler, Kenneth S., Kristen C. Jacobson, Carol A. Prescott, and Michael C. Neale. 2003. "Specificity of Genetic and Environmental Risk Factors for Use and Abuse/dependence of Cannabis, Cocaine, Hallucinogens, Sedatives, Stimulants, and Opiates in Male Twins." *The American Journal of Psychiatry* 160 (4): 687–95.

Kreibig, Sylvia D. 2010. "Autonomic Nervous System Activity in Emotion: A Review." *Biological Psychology* 84 (3): 394–421.

Levenson, Robert W. 2014. "The Autonomic Nervous System and Emotion." *Emotion Review: Journal of the International Society for Research on Emotion* 6 (2). SAGE Publications: 100–112.

Logothetis, Nikos K. 2008. "What We Can Do and What We Cannot Do with fMRI." *Nature* 453 (7197). Nature Publishing Group: 869.

Maschke, M., M. Schugens, K. Kindsvater, J. Drepper, F. P. Kolb, H-C Diener, I. Daum, and D. Timmann. 2002. "Fear Conditioned Changes of Heart Rate in Patients with Medial Cerebellar Lesions." *Journal of Neurology, Neurosurgery, and Psychiatry* 72 (1): 116–18.

Office of the Surgeon General (2016). Facing Addiction in America:TheSurgeon General's Report on Alcohol, Drugs, and Health. Washington, DC: US Dept .of Health and Human Services. http://addiction.surgeongeneral.gov. Accessed November 29, 2016

Robinson, Terry E, Berridge, Kent C. Review. 2008. The incentive sensitization theory of addiction: some current issues. *Philos Trans R Soc Lond B Biol Sci*. 363(1507): 3137-46.

Russell, James A. 1980. "A Circumplex Model of Affect." *Journal of Personality and Social Psychology* 39 (6): 1161–78.

Smith, Kyle S., Kent C. Berridge, and J. Wayne Aldridge. 2011. "Disentangling Pleasure from Incentive Salience and Learning Signals in Brain Reward Circuitry." *Proceedings of the National Academy of Sciences of the United States of America* 108 (27): E255–64.

Snow, Wanda M., Brenda M. Stoesz, and Judy E. Anderson. 2014. "The Cerebellum in Emotional Processing: Evidence from Human and Non-Human Animals." Aims Press. http://www.aimspress.com/article/10.3934/Neuroscience.2014.1.96.

Stone, Arthur A., and Saul Shiffman. 1994. "Ecological Momentary Assessment (EMA) in Behavorial Medicine." *Annals of Behavioral Medicine: A Publication of the Society of Behavioral Medicine*. Lawrence Erlbaum. http://psycnet.apa.org/psycinfo/1995-10701-001.

Styliadis, Charis, Andreas A. Ioannides, Panagiotis D. Bamidis, and Christos Papadelis. 2015. "Distinct Cerebellar Lobules Process Arousal, Valence and Their Interaction in Parallel Following a Temporal Hierarchy." *NeuroImage* 110 (April): 149–61.

# Chapter 5

# Publications

## 1. Manuscript in preparation concerning the findings reported in the present dissertation

A manuscript including the he findings reported in Chapter 2 and Chapter 3 is currently prepared for submission.

## 2. Other manuscripts in preparation

During the course of my Ph.D. program, I contributed to other research projects that were not strictly germane to this dissertation. Two manuscripts reporting the relative findings are in preparation.

2.1. **De Pirro S**, Parkinson J, Badiani A, The effect of alcohol on sense of agency: an intentional binding study on healthy subjects

2.2. **De Pirro S**, Martinez Cornelio P, Badiani A, Subramanian S, The effect of alcohol on sense of agency in touchless system interactions.

2.3. **De Pirro S**, Badiani A, Temporal patterns of heroin and cocaine use in human co-abusers.

## 3. Published papers

In the last two years I co-authored the following two papers reporting findings not related to the present dissertation (see reprints at the end of the Dissertation).

**3.1.** Badiani A, Boden JM, **De Pirro S**, Fergusson DM, Horwood LJ, Harold GT (2015) Tobacco smoking and cannabis use in a longitudinal birth cohort: Evidence of reciprocal causal relationships. Drug Alcohol Depend 150:69-76.

**3.2.** Martinez-Cornelio P, **De Pirro S**, Thanh Vi C, Subramanian S (2017) Agency in Mid-air interface. In Proceedings of the ACM CHI Conference on Human Factors in Computing Systems (CHI 2017, Denver, USA).

# Reprints

Drug and Alcohol Dependence 150 (2015) 69–76

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

# Tobacco smoking and cannabis use in a longitudinal birth cohort: Evidence of reciprocal causal relationships☆

Aldo Badiani[a], Joseph M. Boden[b,*], Silvana De Pirro[a], David M. Fergusson[b], L. John Horwood[b], Gordon T. Harold[c]

[a] Sussex Addiction Research and Intervention Centre (SARIC), School of Psychology, University of Sussex, Brighton, United Kingdom
[b] Christchurch Health and Development Study, University of Otago, Christchurch School of Medicine and Health Sciences, PO Box 4345, Christchurch, New Zealand
[c] Rudd Centre for Adoption Research and Practice, School of Psychology, University of Sussex, and Institute of Genetic, Neurobiological and Social Foundations of Child Development, Tomsk State University, Brighton, United Kingdom

ARTICLE INFO

*Article history:*
Received 4 December 2014
Received in revised form 27 January 2015
Accepted 12 February 2015
Available online 23 February 2015

*Keywords:*
Tobacco
Cannabis
Longitudinal study
Structural equation Modelling

ABSTRACT

*Background:* There is evidence of associations between tobacco and cannabis use that are consistent with both a classical stepping-stone scenario that posits the transition from tobacco use to cannabis use ('gateway' effect of tobacco) and with the reverse process leading from cannabis use to tobacco abuse ('reverse gateway' effect of cannabis). The evidence of direct causal relationships between the two disorders is still missing.

*Methods:* We analysed data from the Christchurch Health and Development Study (CHDS) longitudinal birth cohort using advanced statistical modelling to control for fixed sources of confounding and to explore causal pathways. The data were analysed using both: (a) conditional fixed effects logistic regression modelling; and (b) a systematic structural equation modelling approach previously developed to investigate psychiatric co-morbidities in the same cohort.

*Results:* We found significant ($p < 0.05$) associations between the extent of cannabis use and tobacco smoking and vice versa, after controlling for non-observed fixed confounding factors and for a number of time-dynamic covariate factors (major depression, alcohol use disorder, anxiety disorder, stressful life events, deviant peer affiliations). Furthermore, increasing levels of tobacco smoking were associated with increasing cannabis use ($p = 0.02$) and vice versa ($p < 0.001$) over time.

*Conclusions:* Our results lend support to the notion of both of 'gateway' and 'reverse gateway' effects. That is, the association between tobacco and cannabis use arises from a reciprocal feedback loop involving simultaneous causation between tobacco use disorder and cannabis use disorder.



## 1. Introduction

Tobacco and cannabis are two of the most abused recreational substances worldwide, ranking second and third in prevalence of use after alcohol (Degenhardt et al., 2008). Both tobacco and cannabis are mostly taken via smoking (Agrawal et al., 2012), and the two substances are often co-administered in the form of 'joints' or 'blunts' (cannabis rolled in cigar paper; Ream et al., 2008). Furthermore, many tobacco and cannabis users are co-users; that is, they use the two substances independently of each other either in distinct occasions or in a sequence (e.g., Mayet et al., 2011; Richter et al., 2004).

A multi-criteria analysis of drug harm (Nutt et al., 2010) indicates that tobacco and cannabis are among the four most damaging recreational substances in terms of direct and indirect economic costs to society. Furthermore, it has been proposed that tobacco and cannabis may serve as gateway drugs, leading to the use and abuse of other substances (Anthony, 2012). Gateway theory has been the subject of some controversy in the literature, having been criticized in terms of both drug sequence and causal modelling (Baumrind, 1983; Degenhardt et al., 2009, 2010). However, it is clear that a better understanding of the factors involved in initiating tobacco and cannabis use may shed considerable light on the factors responsible for their use.

Tobacco users who are also cannabis users are more likely to be daily smokers and develop dependence than non-cannabis users

☆ Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:10.1016/j.drugalcdep.2015.02.015.

* Corresponding author. Tel.: +64 3 372 0406; fax: +64 3 372 0407.
*E-mail address:* joseph.boden@otago.ac.nz (J.M. Boden).

http://dx.doi.org/10.1016/j.drugalcdep.2015.02.015
0376-8716/

(Agrawal et al., 2011; Degenhardt et al., 2010; Korhonen et al., 2008, 2010; Timberlake et al., 2007). On the other hand, tobacco smokers who experiment with cannabis are more likely to progress to full-blown cannabis abuse than non-smokers (Ream et al., 2008; Timberlake et al., 2007). Using data collected from the National Household Survey on Drug Use and Health (NSDUH) in 2009, Agrawal et al. (2012) estimated that the probability to develop a cannabis use disorder was more than eight times greater in tobacco users than in non-users and that the probability to develop nicotine dependence in cannabis users was more than three-fold that of non-users. These findings are consistent both with the classical stepping-stone scenario that posits the transition from tobacco use to cannabis use and with the reverse process leading from cannabis use to tobacco abuse (reverse gateway effect of cannabis; Patton et al., 2005; Timberlake et al., 2007; Viveros et al., 2006). Indeed, there are at least three possible explanations for the comorbidity of tobacco and cannabis use disorders. First, it is possible to hypothesize the existence of common or correlated genetic and/or environmental factors that predispose the individual to both substance use disorders. A second possibility is that the association is caused by tobacco acting as a gateway drug to cannabis or vice versa. Finally, it is possible that the associations arise from a reciprocal feedback loop involving simultaneous causation between tobacco use disorder and cannabis use disorder.

Previous studies aimed at investigating these different possibilities have not been conclusive. Mayet et al. (2011), for example, used a homogenous Markov multi-state model to analyze data from a repeated cross-sectional survey to estimate the prevalence of tobacco and cannabis use disorders and their relationship. Their findings were compatible with a process mixing the gateway theory, the reverse gateway theory, and the route of administration model (Agrawal and Lynskey, 2009; Prince van Leeuwen et al., 2011). Thus, the authors concluded that longitudinal studies were necessary to explore the causal relationship between tobacco use disorder and cannabis use disorder. The need of longitudinal studies has also been stressed by Agrawal et al. (2012, 2011).

In the present study, we used data from a 35-year longitudinal study of a New Zealand birth cohort to explore the causal relationships between tobacco use and cannabis use on the basis of the prevalence and frequency of use at five time periods (ages 18, 21, 25, 30 and 35 years). The data were analysed using the same analytic approach previously developed to study the associations between major depression and both alcohol use disorder (Fergusson et al., 2009) and tobacco use (Boden et al., 2010), and between internalizing disorders and substance use disorders (Fergusson et al., 2011). This analytic method incorporates: (a) the use of conditional fixed-effects regression models, augmented by time-dynamic covariate factors, to control for non-observed sources of confounding (Hamerle and Ronning, 1995; Hausman et al., 1984; Judge et al., 1980); and (b) structural equation modelling. This combination of analytic approaches allows inferences concerning possible causal associations between cannabis use and tobacco smoking, and permits examination of the likely direction of causality in the associations between cannabis use and tobacco smoking.

## 2. Methods

### 2.1. Participants

Data were gathered during the course of the Christchurch Health and Development Study (CHDS), a study of a birth cohort of 1265 children (635 males, 630 females) born in the Christchurch (New Zealand) urban region in mid-1977. The cohort has been studied at birth, 4 months, 1 year and annually to age 16 years, and again at ages 18, 21, 25, 30, and 35 years (Fergusson and Horwood, 2001; Fergusson et al., 1989). All study information was collected on the basis of signed consent from study participants and is fully confidential, and is approved by the Canterbury (NZ) Ethics Committee.

### 2.2. Frequency of cannabis use (ages 17–18, 20–21, 24–25, 29–30, and 34–35 years)

At each assessment at ages 18, 21, 25, 30 and 35 years, cohort members were asked about the frequency with which they had used cannabis over the twelve-month period prior to the assessment. For the purposes of this analysis, the frequency data were classified using a three-level variable with the following class intervals: (i) no cannabis use; (ii) >0 times and <1 time per week, and (iii) ≥1 time per week. While these class intervals are somewhat arbitrary, it should be noted that, consistent with previous research (Fergusson and Horwood, 2000; Fergusson et al., 2002), experimentation with alternative classifications produced essentially the same conclusions to those reported here.

### 2.3. Tobacco smoking (ages 18, 21, 25, 30, and 35 years)

At each assessment at ages 18, 21, 25, 30 and 35 years, cohort members were asked about the frequency with which they currently smoked cigarettes. For the purposes of this analysis, the smoking frequency data were classified using a three-level variable with the following class intervals: (i) no tobacco smoking, (ii) >0 cigarettes and <10 cigarettes per day, and (iii) ≥10 cigarettes per day. As with the cannabis frequency data described above, the use of alternative classifications produced similar conclusions to those reported here.

### 2.4. Time-dynamic covariate factors (ages 18, 21, 25, 30 and 35 years)

In order to control for the effects of possible comorbid mental health and substance use disorders and the effects of stressful life events in the analyses, five time-dynamic covariate factors were obtained from the study database. These included: (a) concurrent DSM-IV major depression; (b) concurrent DSM-IV alcohol use disorder; (c) concurrent DSM-IV anxiety disorder; (d) a count measure of the number of stressful life events experienced during the twelve months prior to each assessment; and (e) a measure of the number of cohort members' deviant peers who either used illicit drugs, or were in trouble in the law. Further details of these measures are given in the Online Supplement.

### 2.5. Statistical analyses

*Associations between frequency of cannabis use and frequency of tobacco smoking:* In the first stage of the analyses, the pooled associations between the measures of the frequency of cannabis use and tobacco smoking at ages 18, 21, 25, 30, and 35 years were estimated using Generalized Estimating Equation (GEE) methods (Liang and Zeger, 1986; Zeger and Liang, 1986). Two models were fitted: one in which cannabis use predicted tobacco smoking; and a second model in which tobacco smoking predicted cannabis use. In order to address issues of non-linear trend in each of the predictors, effect proportional scoring methods were used. Specifications of these models are given in Supplementary material[1].

*Fixed effects model for covariate adjustment:* To adjust the associations between cannabis use and tobacco smoking for both: (a) non-observed fixed sources of confounding; and (b) observed time-dynamic covariate factors, conditional fixed effects logistic regression models were fitted to the joint data for each prediction model (cannabis use predicting tobacco smoking; tobacco smoking predicting cannabis use) over the measurement periods (Hamerle and Ronning, 1995; Hausman et al., 1984; Judge et al., 1980). See the Supplementary material[2] for a detailed description of the basis for the fixed effects modelling and model specification.

*Structural equation modelling of reciprocal causal pathways:* To examine the possibility of a reciprocal causal relationship in which cannabis use led to increased risks of tobacco smoking, and tobacco smoking led to increased risks of cannabis use, a structural equation model was fitted to the variance-covariance matrix of the repeated measures of cannabis use and tobacco smoking at each assessment (ages 18, 21, 25, 30, and 35 years), using Mplus and weighted least squares estimation. An example of the reciprocal causal model is displayed in Fig. 1. The model assumes that the reported frequency of tobacco use at these time periods ($t = 1$ to 5) is influenced by fixed sources of variation ($T$), which are constant over time, and by time-dynamic sources of variation ($U_T$). Likewise, the reported frequency of cannabis use is influenced by fixed sources of variation (C) and by time-dynamic sources of variation ($U_C$). The model allows the fixed factors $T$ and $C$ to be correlated. The model also assumes that $U_T$ and $U_C$ are linked by autoregressive processes in which past frequencies predict future frequencies. Finally, the model assumes that $U_T$ and $U_C$ are reciprocally related at $t = 2, 3, 4$, or 5, so that current $U_T$ influences current $U_C$ and vice versa, with these reciprocal effects assumed to be constant over time. Further details of the model assumptions and model fitting are provided in the Supplementary material[3].

[1] Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:10.1016/j.drugalcdep.2015.02.015.

[2] Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:10.1016/j.drugalcdep.2015.02.015.

[3] Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:10.1016/j.drugalcdep.2015.02.015.

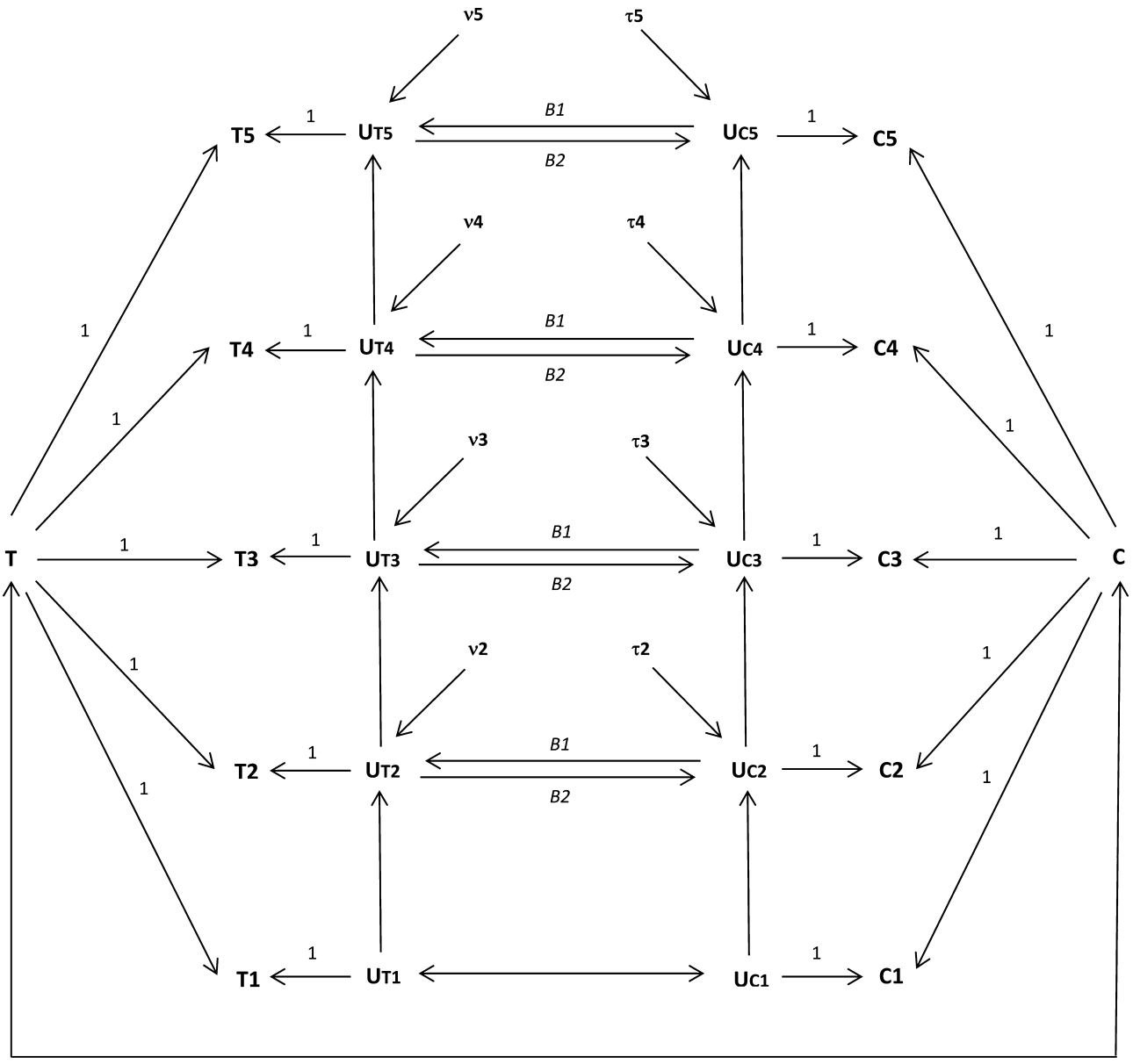



**Fig. 1.** Reciprocal structural model of the associations between cannabis use and tobacco smoking.

### 2.6. Sample size and sample bias

The present analyses were based on 1025 (age 18), 1011 (age 21), 1003 (age 25), 987 (age 30), and 962 (age 35) individuals, representing 76–80% of the original cohort. To examine the effects of sample losses on sample representativeness, the obtained samples with complete data at each age were compared with the remaining sample members on a series of socio-demographic measures collected at birth. These results suggested that there were statistically significant ($p < 0.01$) tendencies for the obtained samples to under-represent individuals from socially-disadvantaged backgrounds. To address this issue, data weighting methods described by Carlin et al. (1999) were used to re-analyze the data, producing the same pattern of results to those reported here, suggesting that the conclusions of this study were unlikely to have been influenced by selection bias.

## 3. Results

### 3.1. Patterns of cannabis use, tobacco smoking and co-use[4]

As shown in Fig. 2 and Tables 1a and 1b, more than half of the cohort (54.5%) reported smoking tobacco and/or cannabis at

[4] Throughout this paper, the term co-use indicates the use of both tobacco and cannabis in distinct occasions by the same individual, and not the co-administration of the two drugs via 'joints' or 'blunts'.

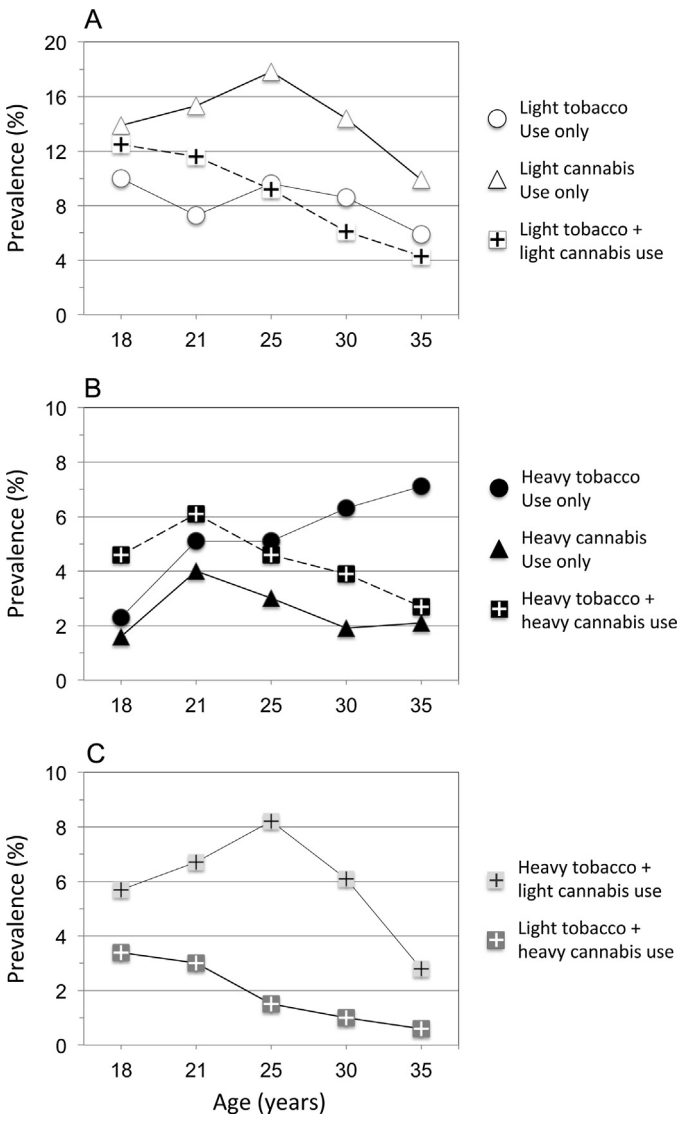



**Fig. 2.** Prevalence of use in the past 12 months at ages 18, 21, 25, 30, and 35 years, for: (A) 'light tobacco smokers-only' (<10 cigarettes per day), 'light cannabis users-only' (<1 time per week), and light co-users; (B) 'heavy tobacco smokers-only' (>10 cigarettes per day), 'heavy cannabis users-only' (≥1 time per week), and heavy co-users; (C) heavy tobacco-light cannabis co-users and light tobacco-heavy cannabis co-users.

age 18 years. The overall number of users peaked at age 21 and progressively decreased, so that at age 35 only 35.3% of the cohort was still smoking tobacco and/or cannabis. Fig. 2 also illustrates the changes in the prevalence of tobacco and cannabis co-use and indicates that the decline in use concerned all types of users and co-users, except those who were 'heavy tobacco smokers-only' (>10 cigarettes per day) or 'heavy cannabis users-only' (≥1 time per week). Fig. 3A shows that among 'tobacco smokers-only' the proportion of heavy users more than tripled from age 18 years to age 35 years. This was not the case for either 'cannabis users-only' or for co-users (Fig. 3B and C).

### 3.2. Associations between cannabis use and tobacco smoking

Table 1a shows the associations between the frequency of cannabis use and the probability of being a tobacco smoker at ages 18, 21, 25, 30, and 35 years. Table 1b shows the frequency of tobacco smoking and the probability of using cannabis at ages 18, 21, 25, 30, and 35 years. The data in both tables were analysed using a random effects GEE model to estimate the associations between: (i) cannabis use and tobacco smoking, and (ii) tobacco smoking and cannabis use. Both analyses show the presence of strong linear associations ($p < 0.0001$) between the extent of cannabis/tobacco use and the probabilities of tobacco smoking/cannabis use, as indicated by the relative OR's.

**Table 1a**
Associations between frequency of cannabis use and probability of tobacco smoking, ages 18, 21, 25, 30, and 35 years.

| Age | Level of cannabis use | *n* | % reporting tobacco smoking |
|---|---|---|---|
| 18 | None | 598 | 22.1 |
| | <Weekly | 329 | 56.5 |
| | ≥Weekly | 98 | 83.7 |
| 21 | None | 538 | 23.4 |
| | <Weekly | 340 | 54.4 |
| | ≥Weekly | 133 | 69.2 |
| 25 | None | 559 | 26.3 |
| | <Weekly | 353 | 49.3 |
| | ≥Weekly | 91 | 67.0 |
| 30 | None | 657 | 21.5 |
| | <Weekly | 262 | 45.0 |
| | ≥Weekly | 68 | 72.1 |
| 35 | None | 747 | 16.7 |
| | <Weekly | 163 | 41.7 |
| | ≥Weekly | 52 | 61.5 |
| Population-averaged rates | % | OR (95% CI) | |
| None | 21.7 | 1 (–) | |
| <Weekly | 49.9 | 5.30 (4.24–6.62) | |
| ≥Weekly | 67.6 | 28.22 (18.17–43.82) | |

### 3.3. Adjustments for confounding

Tables 2a and 2b show the results of analyses controlling for both (i) non-observed fixed confounding factors; and (ii) a number of time-dynamic covariate factors, including: major depression; alcohol use disorder; anxiety disorder; stressful life events; and

**Table 1b**
Associations between frequency of tobacco smoking and probability of cannabis use, ages 18, 21, 25, 30, and 35 years.

| Age | Level of cigarette smoking | *n* | % reporting cannabis use |
|---|---|---|---|
| 18 | None | 625 | 25.4 |
| | <10/day | 271 | 60.1 |
| | 10+/day | 129 | 81.4 |
| 21 | None | 608 | 37.2 |
| | <10/day | 221 | 66.5 |
| | 10+/day | 182 | 71.4 |
| 25 | None | 621 | 33.7 |
| | <10/day | 203 | 52.7 |
| | 10+/day | 179 | 71.6 |
| 30 | None | 677 | 23.8 |
| | <10/day | 149 | 47.0 |
| | 10+/day | 161 | 61.5 |
| 35 | None | 737 | 15.6 |
| | <10/day | 104 | 45.2 |
| | 10+/day | 121 | 43.8 |
| Population-averaged rates | % | OR (95% CI) | |
| None | 25.7 | 1 (–) | |
| <10/day | 56.3 | 5.51 (4.49–6.76) | |
| 10+/day | 66.7 | 30.57 (20.09–45.60) | |

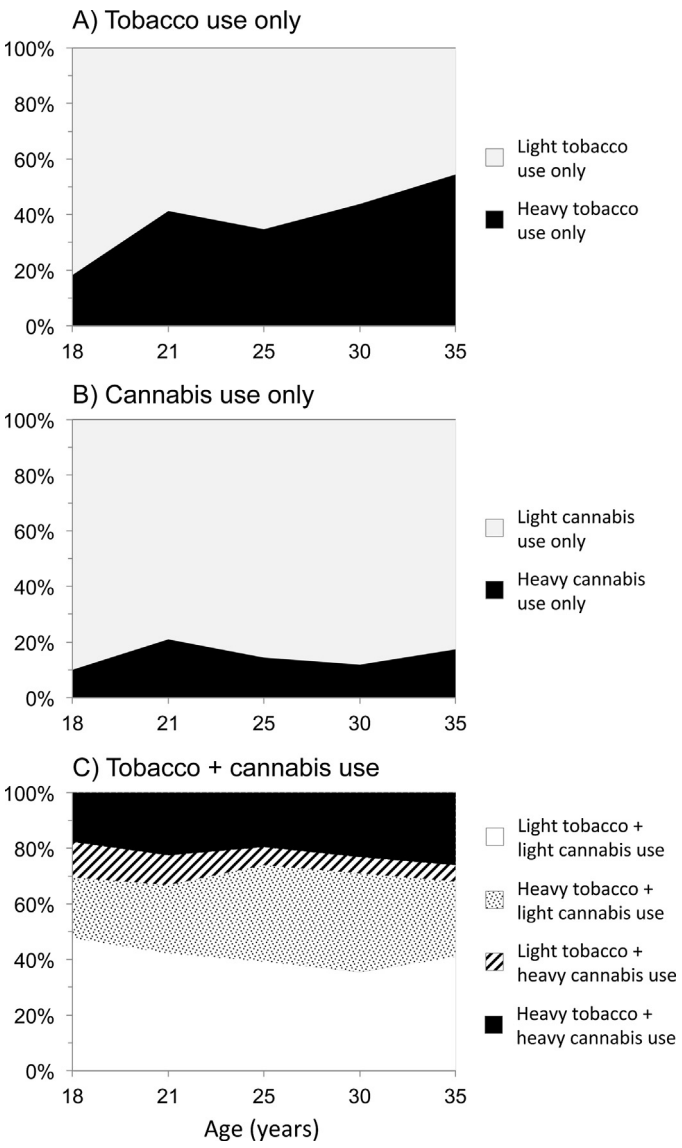



**Fig. 3.** Proportion of light versus heavy use (at ages 18, 21, 25, 30, and 35 years) for: (A) tobacco smokers-only; (B) cannabis users-only; (C) co-users. For the definition of 'light' and 'heavy' see text and Fig. 1.

deviant peer affiliations. Table 2a shows the estimates of the covariate-adjusted coefficients for the prediction of smoking from cannabis use and Table 2b those for the prediction of cannabis use from smoking. Both analyses showed that control for non-observed fixed factors and time-dynamic covariate factors reduced the magnitude of the associations between tobacco smoking and cannabis use. Nonetheless, the adjusted associations remained statistically significant ($p < 0.05$) and substantial, suggesting that the associations between cannabis use and tobacco smoking could not be accounted for by non-observed sources of confounding, or time-dynamic covariate factors.

**Table 2a**
Associations between frequency of cannabis use and probability of tobacco smoking, after adjustment for both: (a) non-observed fixed sources of confounding; and (b) time-dynamic covariate factors.

| | Cigarette smoking |
|---|---|
| Level of cannabis use | OR (95% CI) |
| None | 1 (–) |
| <Weekly | 2.90 (2.34–3.58) |
| ≥Weekly | 8.41 (5.48–12.82) |

Statistically significant ($p < 0.05$) time dynamic covariate factors: major depression; alcohol use disorder; anxiety disorder; life stress; deviant peer affiliation.

**Table 2b**
Associations between frequency of tobacco smoking and probability of cannabis use, after adjustment for both: (a) non-observed fixed sources of confounding; and (b) time-dynamic covariate factors.

| | Cannabis use |
|---|---|
| Level of cigarette smoking | OR (95% CI) |
| None | 1 (–) |
| <10/day | 2.69 (2.21–3.28) |
| 10+/day | 7.24 (4.88–10.76) |

Statistically significant ($p < 0.05$) time dynamic covariate factors: major depression; alcohol use disorder; life stress; deviant peer affiliation.

### 3.4. Testing for reverse causality

The findings in Tables 1a and 1b and 2a and 2b are consistent with three general explanations of the associations between cannabis use and tobacco smoking: (1) cannabis use leads to tobacco smoking; (2) tobacco smoking leads to cannabis use; and (3) there is a reciprocal causal relationship in which both (1) and (2) hold simultaneously. One advantage of longitudinal data is that it is possible to fit structural equation models (SEMs) that permit reciprocal causal pathways (Boden et al., 2010; Fergusson et al., 2009). To examine this possibility, the SEM depicted in Fig. 1 was fitted to the data (see Section 2) using Mplus (the full set of coefficients is available upon request from the corresponding author).

The method of modelling (described in detail in the Supplementary material[5]) estimates two key parameters of interest: (i) the parameter $B_1$ reflecting the causal effect of cannabis use on the level of tobacco smoking, and (ii) the parameter $B_2$ reflecting the causal effect of tobacco smoking on the level of cannabis use.

The model depicted in Fig. 1 was fitted to the repeated measures data on levels of tobacco use and cannabis use over the five time periods from age 18–35. The model showed an excellent fit to the observed data [model $\chi^2(25) = 32.7$, $p = 0.14$; RMSEA = 0.019; CFI = 0.99]. Further, the model showed that after control for non-observed fixed sources of confounding, there was evidence of modest but statistically significant reciprocal associations in which: (i) increasing levels of cannabis use were associated with increasing tobacco smoking ($B_1 = 0.099$; SE = 0.03; 95% CI: 0.040–0.158; $p < 0.001$), and (ii) increasing levels of tobacco smoking were associated with increasing cannabis use ($B_2 = 0.066$; SE = 0.027; 95% CI: 0.013–0.119; $p = 0.02$).

## 4. Discussion

The present study analysed data from the Christchurch Health and Development Study (CHDS) longitudinal birth cohort using advanced statistical modelling to control for fixed sources of confounding, and to explore causal pathways in the associations between cannabis use and tobacco smoking. The findings of these analyses and their implications are outlined below.

### 4.1. Patterns of tobacco smoking and cannabis use

More than half of the CHDS cohort reported using tobacco and/or cannabis at age 18 years, with an overall prevalence of 54.5%. At this age, most of these users (regardless of the frequency of use) were co-users. This pattern is consistent with previous studies

[5] Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:10.1016/j.drugalcdep.2015.02.015.

investigating a similar age bracket (e.g., Agrawal et al., 2011). The overall prevalence of use peaked at age 21 and then progressively decreased to 35.3% by age 35 years.

However, age-dependent changes in the prevalence of use were very different as a function of drug and of intensity of use. The prevalence of use at age 35 years was in fact half that observed at age 18 years for light (<weekly) and heavy (≥weekly) cannabis users (−47.5% and −43.75%, respectively), and for light tobacco smokers (<10 cigarettes per day; −59.1%). In contrast, the prevalence of heavy tobacco smoking at age 35 years was exactly the same observed at age 18 years. More specifically, there was an age-related decline for all types of users and co-users, except for those who were 'heavy tobacco smokers-only' or 'heavy cannabis users-only' (see Fig. 1), indicating the emergence of more selective drug preferences. This was particularly true of heavy tobacco smokers-only, whose prevalence increased more than 200% from age 18 years to age 35 years (see Figs. 1C and 2A) whereas for heavy cannabis users-only the increase was only by 31%, confirming that the addictive potential of tobacco is much greater than that of cannabis, whereas the probability of remission from dependence is much lower (Lopez-Quintero et al., 2011a, 2011b).

An important aspect of the CHDS birth cohort is that the prevalence of use for cannabis-only was comparable to that for tobacco-only at ages 18 and 35, a pattern that is characteristic of New Zealand and Australia (e.g., Degenhardt et al., 2008; Swift et al., 2012) and that stands in sharp contrast with that observed in most other countries, where the prevalence of tobacco use is much higher than that of cannabis (e.g., Agrawal et al., 2012; Degenhardt et al., 2008). The similarity in the prevalence of tobacco and cannabis use is ideally suited to investigate the reciprocal influences between the two conditions.

### 4.2. Reciprocal influences between tobacco smoking and cannabis use

In agreement with previous studies (Agrawal et al., 2011; Degenhardt et al., 2010; Korhonen et al., 2008, 2010; Ream et al., 2008; Timberlake et al., 2007), we found a significant association between tobacco smoking and cannabis use. Light cannabis users had approximately five times greater odds of being tobacco smokers than non-users. Also the odds of light tobacco smokers of being cannabis users were about five-fold those of non-smokers. The odds of co-use greatly increased in heavy users. Both heavy tobacco smokers and heavy cannabis users were about thirty times more likely to co-use the other substance than the respective non-users.

One possible explanation for these associations is that they arose because of common confounding factors, including non-observed fixed effects as well as time dynamic covariate factors, such as concurrent psychiatric disorders (major depression, alcohol use disorder, and anxiety disorder), stressful life events and deviant peer affiliations. However, significant and robust associations remained evident even after adjustment for both non-observed fixed confounding and time-dynamic covariate factors, suggesting that the associations between cannabis use and tobacco use could not be explained by confounding. After adjustment, light users of either substance had approximately three times the adjusted odds of also using the other substance relative to the respective non-users. In heavy users of either substance the adjusted odds were seven to eight times greater than those of the respective non-users. This pattern of findings suggests a possible reciprocal causal association in which cannabis use increases the risk of tobacco use, and vice-versa.

To explore the possible pathways between tobacco smoking and cannabis use, structural equation modelling was used to fit a reciprocal causation model. This analysis suggested that the best-fitting model was one in which there was a bidirectional association between tobacco smoking and cannabis smoking, in which: (i) the use of one substance leads to the use the other substance; and (ii) the greater the intensity of use of one substance the greater the intensity of use of the other substance.

To the best of our knowledge, this is the first longitudinal study to investigate the reciprocal causal relationships between tobacco smoking and cannabis use. Our findings confirm and extend those other longitudinal studies concerned with unidirectional influences of tobacco use on cannabis use or vice versa. A 10-year cohort study conducted by Patton et al. (2005) investigated the role of cannabis use in the later initiation of tobacco use and progression to dependence. They found that at least one report of weekly cannabis use in the teens was associated to a more than eight-fold increase in the odds of later initiation of tobacco use whereas daily cannabis use at age 21 years was associated to a more than three-fold increase in the odds of progressing to tobacco dependence. A more recent longitudinal study by Prince van Leeuwen et al. (2014) examined whether tobacco use during adolescence affected the likelihood to abuse cannabis. They found that both early-onset tobacco use and continued tobacco use in adolescence doubled the likelihood of developing a cannabis use disorder.

The findings reported here have important implications for the 'gateway' hypotheses, which posits a progression in drug use, beginning with tobacco and alcohol, moving on to cannabis, and then to other illicit drugs (Botvin et al., 2000; Kandel and Faust, 1975; Kandel, 1984; Kandel et al., 1992; Lynskey et al., 2003). The nature of these "gateway" effects is a matter of some debate (Degenhardt et al., 2010; Fergusson et al., 2006; Kandel et al., 2006; MacCoun, 2006; Morral et al., 2002; Prince van Leeuwen et al., 2011; Vanyukov et al., 2012). In particular, it is not clear whether the predictive association between cannabis and other illicit drug use is causal or reflects confounding factors (Fergusson et al., 2006; Hall and Lynskey, 2005; Kandel and Faust, 1975; Kandel, 1984; Kandel et al., 2006; MacCoun, 2006; Morral et al., 2002). Furthermore, there is data suggesting a "reverse gateway" effect of cannabis use on tobacco use (Patton et al., 2005; Viveros et al., 2006). The systematic structural equation model used in the present study indicates the simultaneous occurrence of 'gateway' and 'reverse gateway' effects. That is, the association between tobacco and cannabis use arises from a reciprocal feedback loop involving simultaneous causation between tobacco use and cannabis use.

With the present study adding to the growing evidence concerning a possible gateway role of tobacco in linkages with cannabis and other illicit drugs, further questions arise concerning the mechanisms underpinning these linkages. The route of administration model (Agrawal and Lynskey, 2009) would suggest that the origins of these linkages are either physiological or cultural in nature, in which the use of either tobacco or cannabis is increased by: (a) the act of smoking one or the other substance causes aero-respiratory changes; and/or (b) social and cultural practices in which tobacco and cannabis are consumed simultaneously (via "joints" or "blunts"). Further research is necessary to better distinguish between these accounts of the linkages between tobacco and cannabis use.

A further possible explanation for the observed associations between cannabis and tobacco is the common liability model (Prince van Leeuwen et al., 2011), in which genetic and individual factors are thought to increase the risk of the use of multiple substances. However, it would seem to be the case that such factors should have been accounted for in the present analyses by: (a) the use of conditional fixed-effects models; and (b) the correlated latent indices for cannabis and tobacco in the structural models.

### 4.3. Limitations of the study

It is important to recognize that the conclusions drawn in this analysis rely on some underlying assumptions. The most pervasive

of these assumptions is that the pattern of causal relationships can be modelled as a stable causal process that was operative throughout the course of this study. This is clearly a strong assumption, but it is essential for both the fixed-effects and reciprocal-causes models. Additional research is required to verify whether our assumption is correct. It is also possible that our structural equations do not adequately reflect the complexity of all the factors at play, an issue that can be addressed only by further investigations based on models partly or radically different from the one used here. Finally, it should also be noted that specific instances of co-use of cannabis and tobacco (e.g. rolled together in "joints") was not measured in the present study.

## Author disclosures

*Role of funding source*

This research was funded by grants from the Health Research Council of New Zealand (Grant No: 11/792), the National Child Health Research Foundation, the Canterbury Medical Research Foundation, and the New Zealand Lottery Grants Board. Authors AB and SDP were supported by funding from the Sussex Addiction Research and Intervention Centre (SARIC), School of Psychology, University of Sussex. None of the funding sources played any role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

## Contributors

The collaboration between research centres was established by AB, DMF, LJH, and GTH. AB, JMB, and SDP performed literature searches. JMB and LJH analysed the data. AB and DMF designed the study and reviewed the analyses. JMB, DMF, and LJH collected the data. All authors contributed to the writing of the draft article, and approved of the final manuscript.

## Conflict of interest

No conflict declared.

## Acknowledgements

This research was funded by grants from the Health Research Council of New Zealand, the National Child Health Research Foundation, the Canterbury Medical Research Foundation, and the New Zealand Lottery Grants Board.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.drugalcdep.2015.02.015.

## References

Agrawal, A., Budney, A.J., Lynskey, M.T., 2012. The co-occurring use and misuse of cannabis and tobacco: a review. Addiction 107, 1221–1233.
Agrawal, A., Lynskey, M.T., 2009. Tobacco and cannabis co-occurrence: does route of administration matter? Drug Alcohol Depend. 99, 240–247.
Agrawal, A., Scherrer, J.F., Lynskey, M.T., Sartor, C.E., Grant, J.D., Haber, J.R., Madden, P.A., Jacob, T., Bucholz, K.K., Xian, H., 2011. Patterns of use, sequence of onsets and correlates of tobacco and cannabis. Addict. Behav. 36, 1141–1147.
Anthony, J.C., 2012. Steppingstone and gateway ideas: a discussion of origins, research challenges, and promising lines of research for the future. Drug Alcohol Depend. 123 (Suppl. 1), S99–S104.
Baumrind, D., 1983. Specious causal attributions in the social sciences: the reformulated stepping-stone theory of heroin use as exemplar. J. Pers. Soc. Psychol. 45, 1289–1298.
Boden, J.M., Fergusson, D.M., Horwood, L.J., 2010. Cigarette smoking and depression: tests of causal linkages using a longitudinal birth cohort. Br. J. Psychiatry, 440–446.
Botvin, G.J., Griffin, K.W., Diaz, T., Scheier, L.M., Williams, C., Epstein, J.A., 2000. Preventing illicit drug use in adolescents: long-term follow-up data from a randomized control trial of a school population. Addict. Behav. 25, 769–774.
Carlin, J.B., Wolfe, R., Coffey, C., Patton, G.C., 1999. Tutorial in biostatistics, analysis of binary outcomes in longitudinal studies using weighted estimating equations and discrete-time survival methods: prevalence and incidence of smoking in an adolescent cohort. Stat. Med. 18, 2655–2679.
Degenhardt, L., Chiu, W.T., Conway, K., Dierker, L., Glantz, M., Kalaydjian, A., Merikangas, K., Sampson, N., Swendsen, J., Kessler, R.C., 2009. Does the 'gateway' matter? Associations between the order of drug use initiation and the development of drug dependence in the National Comorbidity Study Replication. Psychol. Med. 39, 157–167.
Degenhardt, L., Chiu, W.T., Sampson, N., Kessler, R.C., Anthony, J.C., Angermeyer, M., Bruffaerts, R., de Girolamo, G., Gureje, O., Huang, Y., Karam, A., Kostyuchenko, S., Lepine, J.P., Mora, M.E., Neumark, Y., Ormel, J.H., Pinto-Meza, A., Posada-Villa, J., Stein, D.J., Takeshima, T., Wells, J.E., 2008. Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. PLoS Med. 5, 1053–1067.
Degenhardt, L., Dierker, L., Chiu, W.T., Medina-Mora, M.E., Neumark, Y., Sampson, N., Alonso, J., Angermeyer, M., Anthony, J.C., Bruffaerts, R., de Girolamo, G., de Graaf, R., Gureje, O., Karam, A.N., Kostyuchenko, S., Lee, S., Lépine, J.P., Levinson, D., Nakamura, Y., Posada-Villa, J., Stein, D., Wells, J.E., Kessler, R.C., 2010. Evaluating the drug use gateway theory using cross-national data: consistency and associations of the order of initiation of drug use among participants in the WHO World Mental Health Surveys. Drug Alcohol Depend. 108, 84–97.
Fergusson, D.M., Boden, J.M., Horwood, L.J., 2006. Cannabis use and other illicit drug use: testing the cannabis gateway hypothesis. Addiction 101, 556–569.
Fergusson, D.M., Boden, J.M., Horwood, L.J., 2009. Tests of causal linkages between alcohol abuse or dependence and major depression. Arch. Gen. Psychiatry 66, 260–266.
Fergusson, D.M., Boden, J.M., Horwood, L.J., 2011. Structural models of the comorbidity of internalizing disorders and substance use disorders in a longitudinal birth cohort. Soc. Psychiatry Psychiatr. Epidemiol. 46, 933–942.
Fergusson, D.M., Horwood, L.J., 2000. Does cannabis use encourage other forms of illicit drug use? Addiction 95, 505–520.
Fergusson, D.M., Horwood, L.J., 2001. The Christchurch Health and Development Study: review of findings on child and adolescent mental health. Aust. N.Z. J. Psychiatry 35, 287–296.
Fergusson, D.M., Horwood, L.J., Shannon, F.T., Lawton, J.M., 1989. The Christchurch Child Development Study: a review of epidemiological findings. Paediatr. Perinat. Epidemiol. 3, 278–301.
Fergusson, D.M., Horwood, L.J., Swain-Campbell, N.R., 2002. Cannabis use and psychosocial adjustment in adolescence and young adulthood. Addiction 97, 1123–1135.
Hall, W., Lynskey, M., 2005. Is cannabis a gateway drug? Testing hypotheses about the relationship between cannabis use and the use of other illicit drugs. Drug Alcohol Rev. 24, 39–48.
Hamerle, A., Ronning, G., 1995. Panel analysis for qualitative variables. In: Arminger, G., Clogg, C.C., Sobel, M.E. (Eds.), Handbook of Statistical Modeling for the Social and Behavioral Sciences. Plenum Press, New York, NY, pp. 401–451.
Hausman, J., Hall, B.H., Griliches, Z., 1984. Econometric models for count data with an application to the patents—R & D relationship. Econometrica 52, 909–938.
Judge, G.E., Griffiths, W.E., Hill, R.C., Lee, T. (Eds.), 1980. The Theory and Practice of Econometrics. John Wiley & Sons, New York, NY.
Kandel, D., Faust, R., 1975. Sequences and stages in patterns of adolescent drug use. Arch. Gen. Psychiatry 32, 923–932.
Kandel, D.B., 1984. Marijuana users in young adulthood. Arch. Gen. Psychiatry 41, 200–209.
Kandel, D.B., Yamaguchi, K., Chen, K., 1992. Stages of progression in drug involvement from adolescence to adulthood: further evidence for the gateway theory. J. Stud. Alcohol 53, 447–457.
Kandel, D.B., Yamaguchi, K., Klein, L.C., 2006. Testing the gateway hypothesis. Addiction 101, 470–476.
Korhonen, T., Huizink, A.C., Dick, D.M., Pulkkinen, L., Rose, R.J., Kaprio, J., 2008. Role of individual, peer and family factors in the use of cannabis and other illicit drugs: a longitudinal analysis among Finnish adolescent twins. Drug Alcohol Depend. 97, 33–43.
Korhonen, T., Prince van Leeuwen, A., Reijneveld, S.A., Ormel, J., Verhulst, F.C., Huizink, A.C., 2010. Externalizing behavior problems and cigarette smoking as predictors of cannabis use: the TRAILS Study. J. Am. Acad. Child Adolesc. Psychiatry 49, 61–69.
Liang, K.-Y., Zeger, S.L., 1986. Longitudinal data analysis using generalized linear models. Biometrika 73, 13–22.
Lopez-Quintero, C., Hasin, D.S., de Los Cobos, J.P., Pines, A., Wang, S., Grant, B.F., Blanco, C., 2011a. Probability and predictors of remission from life-time nicotine, alcohol, cannabis or cocaine dependence: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Addict. Biol. 106, 657–669.
Lopez-Quintero, C., Pérez de los Cobos, J., Hasin, D.S., Okuda, M., Wang, S., Grant, B.F., Blanco, C., 2011b. Probability and predictors of transition from first use to dependence on nicotine, alcohol, cannabis, and cocaine: results of the National

Epidemiologic Survey on Alcohol and Related Conditions (NESARC). Drug Alcohol Depend. 115, 120–130.
Lynskey, M.T., Heath, A.C., Bucholz, K.K., Slutske, W.S., Madden, P.A.F., Nelson, E.C., Statham, D.J., Martin, N.G., 2003. Escalation of drug use in early-onset cannabis users vs co-twin controls. JAMA 289, 427–433.
MacCoun, R., 2006. Competing accounts of the gateway effect: the field thins, but still no clear winner. Addiction 101, 470–476.
Mayet, A., Legleye, S., Chau, N., Falissard, B., 2011. Transitions between tobacco and cannabis use among adolescents: a multi-state modeling of progression from onset to daily use. Addict. Behav. 36, 1101–1105.
Morral, A.R., McCaffrey, D.F., Paddock, S.M., 2002. Reassessing the marijuana gateway effect. Addiction 97, 1493–1504.
Nutt, D.J., King, L.A., Phillips, L.D., Independent Scientific Committee on Drugs, 2010. Drug harms in the UK: a multicriteria decision analysis. Lancet 376, 1558–1565.
Patton, G.C., Coffey, C., Carlin, J.B., Sawyer, S.M., Lynskey, M., 2005. Reverse gateways? Frequent cannabis use as a predictor of tobacco initiation and nicotine dependence. Addiction 100, 1518–1525.
Prince van Leeuwen, A., Creemers, H.E., Verhulst, F.C., Vollebergh, W.A.M., Ormel, J., van Oort, F.V.A., Huizink, A.C., 2014. Legal substance use and the development of a DSM-IV cannabis use disorder during adolescence: the TRAILS study. Addiction 109, 303–311.
Prince van Leeuwen, A., Verhulst, F.C., Reijneveld, S.A., Vollebergh, W.A., Ormel, J., Huizink, A.C., 2011. Can the gateway hypothesis, the common liability model and/or, the route of administration model predict initiation of cannabis use during adolescence? A survival analysis—the TRAILS study. J. Adolesc. Health Care 48, 73–78.
Ream, G.L., Benoit, E., Johnson, B.D., Dunlap, E., 2008. Smoking tobacco along with marijuana increases symptoms of cannabis dependence. Drug Alcohol Depend. 95, 199–208.
Richter, K.P., Kaur, H., Resnicow, K., Nazir, N., Mosier, M.C., Ahluwalia, J.S., 2004. Cigarette smoking among marijuana users in the United States. Subst. Abuse 25, 35–43.
Swift, W., Coffey, C., Degenhardt, L., Carlin, J.B., Romaniuk, H., Patton, G.C., 2012. Cannabis and progression to other substance use in young adults: findings from a 13-year prospective population-based study. J. Epidemiol. Comm. Health 66, e26.
Timberlake, D.S., Haberstick, B.C., Hopfer, C.J., Bricker, J., Sakai, J.T., Lessem, J.M., Hewitt, J.K., 2007. Progression from marijuana use to daily smoking and nicotine dependence in a national sample of U.S. adolescents. Drug Alcohol Depend. 88, 272–281.
Vanyukov, M.M., Tarter, R.E., Kirillova, G.P., Kirisci, L., Reynolds, M.D., Kreek, M.J., Conway, K.P., Maher, B.S., Iacono, W.G., Bierut, L., Neale, M.C., Clark, D.B., Ridenour, T.A., 2012. Common liability to addiction and gateway hypothesis: theoretical, empirical and evolutionary perspective. Drug Alcohol Depend. 123, S3–S17.
Viveros, M.P., Marco, E.M., File, S.E., 2006. Nicotine and cannabinoids: parallels, contrasts and interactions. Neurosci. Biobehav. Rev. 30, 1161–1181.
Zeger, S.L., Liang, K.-Y., 1986. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42, 121–130.

# Agency in Mid-air Interfaces

**Patricia Cornelio Martinez[1], Silvana De Pirro[2], Chi Thanh Vi[3], Sriram Subramanian[1]**
[1]INTERACT LAB, [2]SARIC, [3]SCHI LAB
University of Sussex, UK
[P.Cornelio-Martinez, S.DePirro, C.Vi, Sriram]@sussex.ac.uk

**ABSTRACT**
Touchless interfaces allow users to view, control and manipulate digital content without physically touching an interface. They are being explored in a wide range of application scenarios from medical surgery to car dashboard controllers. One aspect of touchless interaction that has not been explored to date is the Sense of Agency (SoA). The SoA refers to the subjective experience of voluntary control over actions in the external world. In this paper, we investigated the SoA in touchless systems using the intentional binding paradigm. We first compare touchless systems with physical interactions and then augmented different types of haptic feedback to explore how different outcome modalities influence users' SoA. From our experiments, we demonstrated that an intentional binding effect is observed in both physical and touchless interactions with no statistical difference. Additionally, we found that haptic and auditory feedback help to increase SoA compared with visual feedback in touchless interfaces. We discuss these findings and identify design opportunities that take agency into consideration.

**Author Keywords**
Touchless interfaces; haptics; the sense of agency; intentional binding; gestures.

**ACM Classification Keywords**
H.5.2 User Interfaces: Theory and methods.

**INTRODUCTION**
Recent advances in gesture recognition technologies [31, 40] are driving a new class of interactive systems where a user is able to view, control and manipulate digital content without touching the interface. For example, touchless interactions are being explored as part of medical surgery [51], to design games that benefit children with Autism Spectrum Disorder (ASD) [4], and touchless controllers for car dashboards [2](see Figure 1). There is a strong user appetite for such systems as they are intuitive and enable greater freedom of user-movements.


*CHI 2017,* May 06-11, 2017, Denver, CO, USA
 ISBN 978-1-4503-4655-9/17/05…$15.00
DOI: http://dx.doi.org/10.1145/3025453.3025457

One aspect of touchless interaction that has not been studied is the SoA in such interactions. The SoA can be defined as the feeling of one's voluntary actions causing events in the external world [22] and having the awareness of owning the actions' outcomes. This "Attribution of judgement" allows us to distinguish our actions and their sensory effects from those of other people [25].

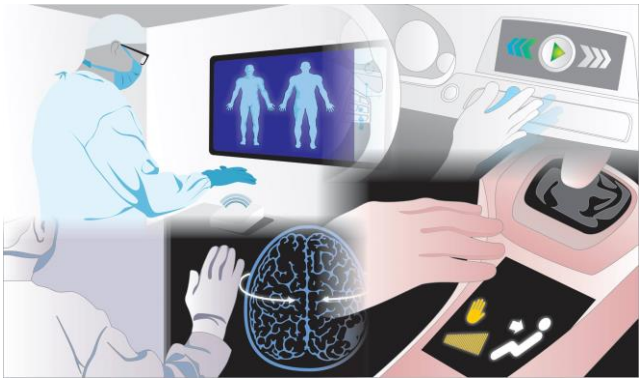


**Figure 1. A mosaic of touchless interactions in surgery and driving scenarios.**

For example, in touchless applications scenarios in which perceiving a responsive system is relevant (e.g. surgery and driving), if users do not experience perception of causation (causal relationship between action and outcome), they could diminish self-attribution of an unfavorable outcome. However, although this perception is independent of correct performance of the device or system (i.e. personal agency), different interaction paradigms can improve this perception in order to enhance user's SoA through a responsive touchless system. Here, we explored these possibilities

To understand users' SoA when interacting with touchless interfaces, we conducted two user studies. Our studies use the intentional binding paradigm, which provides an implicit and quantitative measure of the SoA [23, 43]. In the first experiment, we compared a camera-based button-click gesture with a physical button press, using both visual and auditory feedback. Our results show that both physical and gestural input modalities produce intentional binding. Additionally, we found that participants exhibited significantly more SoA when the touchless input action was accompanied by an auditory outcome rather than a visual outcome. In the second study, we compared the camera-based click gesture both with and without tactile stimuli to examine if haptic feedback can enhance SoA. Our results show that haptic feedback provides higher intentional

binding when gesture-based action precedes it, compared with visual feedback.

The main contributions of our paper are:

- We investigate agency effects for touchless gesture-based interaction.
- We demonstrate by implicit and quantitative metrics that touchless gesture-based input modality could be as responsive as a physical touch-based input modality.
- We show that auditory and haptic feedback help to increase user's SoA in touchless interaction compared with visual feedback.
- We discuss our findings and identify design opportunities that take agency into consideration.

## RELATED WORK

The background for our work comes from the areas of experimental paradigms of agency in neuroscience, agency experiments within HCI and touchless interaction systems. Next, we will provide an overview of these areas.

### The Sense of Agency

There is growing interest in investigating an important aspect of self-consciousness that concerns the awareness of being in control of our own actions: the Sense of Agency (SoA), which refers to "Attribution of judgement". Georgieff and Jeannerod defined this phenomenon as a "Who" system that permits the identification of the agent of an action and thus differentiates the self from external agents [18]. This concept has been studied extensively in cognitive neuroscience to analyze how the deficit in people's sense of agency is a consequence of some diseases or mental disorders like schizophrenia; patients with this disorder do not feel they are in control of their own actions and sometimes their thoughts [39].

Currently, two models explain the origins of agency: prediction and postdiction [59]. According to the predictive model, the SoA is generated by the intention to act, which arises from neural processes that regulate initiation of voluntary motor movement [42]. The postdictive model relies on retrospective reflection, so that the SoA arises after perceiving the action's outcome [34, 62]. Here, the perception of causation (causal relationship between action and outcome) is a result of post-action information.

One example of supportive evidence of the predictive model is the work of Benjamin Libet [30], who studied the timeline regarding brain neural activity and the conscious experience of executing a motor movement. His results suggest that the intention of movement is generated by a brain process over which we have no control, as at that moment we are not consciously aware. However, the subjective experience of free will emerges 200 ms before the actual motor movement. Some researchers have suggested that free will could be described as "free won't" as this process seems to have more to do with the *decision* to execute an action or not, before the action itself [52].

On the other hand, many studies have shown evidence of the postdictive explanation of agency. Johansson et al. [26] observed postdictive influence over subjects' actions based on *choice blindness*. In this study, participants were asked to visually choose one option among others. Then, the experimenters swapped the participants' chosen option with a new one, and presented this new option as their original choice. When participants were asked to explain the reason for their choice, they tried to justify why they chose the swapped option, even though it was clearly different to the original choice.

Another example is the study by Takahata et al. [60] who conducted an experiment where participants were primed with rewarding and punishing outcomes by associating auditory stimuli with positive, neutral and negative monetary outcomes. Their results showed that participants attributed an action to themselves depending on outcome condition; they generally attributed the action to an external factor when its effect produced a negative outcome.

Body ownership also plays an important role in the experience of agency. Participants in [3] falsely attributed an action (speaking) to themselves. The experiment consisted of a virtual reality scene in which participants saw a life-size speaking avatar seen from the first person perspective through a virtual mirror. Participants received thyroid cartilage vibrotactile stimulation synchronized with the avatar's speech. The movements of the virtual body and participant body were also synchronized so that they created the illusion of body ownership.

They demonstrated that participants thought they were speaking the words when they actually were not. In a more recent work they found illusion of agency over walking in seated participants [28]. These findings suggest that people experience SoA even when there is no previous intention to act i.e. in absence of prediction, priming or cause preceding the effect. Although the studies differ in explanations about the initiation of SoA, both models are considered valid [59].

### Agency in HCI

Although the experience of agency is central in cognitive neuroscience, recent research has focused on studying how personal agency changes with use of technology. These studies have opened a new area which aims to explore how "*in-control*" users feel when interacting with an interface, i.e. have the awareness to say, "I am, who is controlling this". McEneaney et al. [37] executed a series of experiments to demonstrate that the experience of agency not only applies for physical situations but also in HCI. They focused on answering: "*Are agency effects observed in desktop computing environments typical of HCI?*"

They based their studies on measuring perception of click-responses through visual stimuli on-screen and auditory feedback to compare human-initiated actions with

computer-controlled actions. Their results showed that an agency effect exists in typical HCI desktop computer environments. This finding supports the claim that user perception of on-screen events depends on agency cues. However, they found that the perception in time of participants differed depending on whether an auditory effect followed a machine or human-initiated click action. Coyle et al. [12] compared a new input modality (Skinput [24]) with physical interaction (button-press) to explore the experience of agency in HCI environments. Skin-based input modality consisted of a piezoelectric microphone on participants' forearm so that a tap on the skin can be recognized as a "button-press" action, preceding an audio feedback in response. The results showed that skin-based input could elicit greater SoA unlike typical keyboard input.

In another example, Limerick et al. [32] explored voice command input. This technique consists of asking participants to say the word "go" as an instruction/action preceding an audio feedback. Their results showed a low SoA in this input modality, suggesting that this low feeling of control contributes to the low uptake of speech interfaces for interactive applications, despite the availability of high accuracy voice recognition techniques (e.g. 97.3% recognition rate). This research suggests that a system that evokes a low sense of agency will discourage users from using it, preventing widespread use of the system. On the other hand, the research of Coyle creates a large opportunity for on-body interaction systems. We need a similar understanding of the SoA for touchless systems in order to improve touchless interface design and thus enable wider uptake of such systems.

### Touchless Systems

Interactive systems that use a touchless approach typically require no physical contact with a surface or object, avoiding the constraints of ordinary interaction paradigms (e.g. mouse and keyboard). These systems often rely on gesture-tracking technologies to detect mid-air gestures. The most common approaches rely on optical technology [61, 68] and electromyography (EMG) [38, 50]. However more recent devices offer higher resolution of gesture-sensing based on radar [31] and sonar [49] technologies.

Taking advantage of its properties, touchless systems are being deployed to perform interactions in many critical situations such as surgery and dashboard control. Touchless manipulation of medical images allows surgeons to maintain the sterile environment required in surgery, without the help of assistants [51]. Another example is driving; today there are many dashboard panels that allow users to control car elements from a distance [2, 29]. The use of gesture recognition and proximity to manipulate car controllers allows the user to release the visual channel, and thus aims to promote a safer driving environment.

Although mid-air gesture-based devices may consist of a wide range of capabilities, most radar, sonar or optical tracking-based gestures typically share common characteristics with mice and tablets. In both devices the main interplay consists of pointing and clicking actions [65]. In these mid-air gesture interaction systems, pointing is represented by hand tracking and clicking is represented by *"activation gestures"* [65], which define the intention to communicate with the system [20]. These gestures must be natural and intuitive, but uncommon, so that they are not performed accidentally [7]. Following this, the user expects a confirmation of the activation, i.e. a perceptible response from the system. This refers to "*system attention*" [5], which is attained with multisensory feedback. Feedback is important in touchless systems as there is no physical contact with an object (e.g. floating images or virtual keyboard). However, it is not necessary to physically touch an object to have the perception of a "button-press" if it is associated with an effect in response.

### Visual, audio and haptic feedback

Touchless interaction can be helped by sensory effects in order for the user to perceive "*system attention*" [5]. This can be achieved by providing users with multisensorial feedback, i.e. visual, auditory and haptic [20]. Freeman and Lantz added light, audio and tactile displays to assist users to know "where to gesture" [16]. Markussen et al. implemented a gestural typing system helped with visual feedback through a virtual keyboard [36]. Liu et al. added visual hand-cursors on-screen to make users know the state of the bare-hand postures and gestures [33]. Wu and Rank explored different audio feedback designs for hand gestures for encouraging immersion in games [66]. In a recent work they found that in-air gestures with responsive audio feedback leads to a higher immersion and enjoyment in video games [67]. Müller et al. developed a technique to "touch" and manipulate sound in mid-air by combining audio, visual and tactile feedback [48].

A common criticism of touchless systems is that users lack haptic feedback for action confirmation. However, mid-air haptic feedback is a recent technique to make the user aware of "*system attention*" in touchless interaction. Airwave [21], UltraHaptics [8] and AIREAL [57] are examples of emerging systems that can provide this missing tactile feedback in mid-air with bare hands. This technology allows users to perceive tactile sensation even in the absence of physical objects. Based on this approach, Monnai et al. proposed a system to interact with floating images, using not only visual feedback (through light beams), but also mid-air haptic feedback through ultrasound in order to create the sensation of touching a virtual screen [41]. In a more recent work, Makino et al. introduced a system to clone real objects into virtual ones. It consisted of floating images that replicate haptic properties of real objects using ultrasound, providing realistic interaction of touch in mid-air without wearable devices [35].

The above examples represent complex systems of touchless input commands with different kinds of feedback.

However, the role of agency during the interaction with these systems has not been investigated. In other words, it is unclear if adding tactile feedback helps user feel SoA when interacting with touchless systems. We believe that agency implication should be considered in touchless interface design in order to improve user involvement, intuitiveness and instinctive sense of control during the interaction.

This is the seventh of Shneiderman's Eight Golden Rules of Interface Design; this rule indicates that interface design should "*support an internal locus of control*"[56] which refers to users' need to feel they are the agents of the system's responses (i.e. "*they are in charge of the system*"). This is a relevant aspect for new application scenarios (e.g. surgery and driving) in which feeling ownership of the outcomes of one's actions is essential. To investigate user's SoA beyond traditional input in these new scenarios, we explored agency by employing the intentional binding paradigm and the Libet clock in a set of input command actions (physical and touchless) and sensory responses that include audio, visual and haptic feedback.

## THE INTENTIONAL BINDING PARADIGM

We used the intentional binding paradigm for our studies. It was developed to provide an implicit and quantitative measure of the SoA [23]. In 2002 Patrick Haggard showed that the perceived time of a voluntary action and its sensory outcomes are shifted towards each other, so that the interval between action and outcome is perceived as shorter than it actually is, leading to a perception of compression of time [23] (see Figure 2 right). As shown in Figure 2 right, the action binding represents the interval between the actual and perceived action; it occurs when the action is perceived to occur later than the moment when it actually did. Similarly, the outcome binding represents the interval between the actual and perceived outcome, it occurs when the sensory effect is perceived earlier than the moment when it actually happened. The sum of these two elements represents a total binding value. Consequently, higher total binding value is related to a higher SoA [12, 14, 45].

According to this method, the action binding and outcome binding can be measured quantitatively. They are calculated from four conditions (see Table 1) consisting of two baseline- (baseline action & baseline outcome) and two active- (active action & active outcome) conditions. As illustrated in Table 1, in the action baseline condition, participants performed the action (physical or touchless) but receive no feedback. In the outcome baseline condition, participants received feedback (visual, auditory or haptic) without performing any action. In the active conditions, both action and outcome occurred. During the task, both actual time (the time logged by the system) and perceived time (reported by the user using a Libet clock) of the action and outcome was recorded. The errors were calculated by the difference between perceived and actual moments of time. Following this, the intentional binding is calculated through the formulas shown in Figure 3 [46].

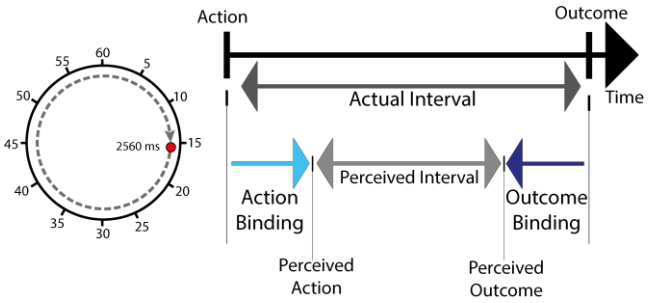



**Figure 2. Illustration of the intentional binding effect (right). The Libet clock (left).**

| Measurement Blocks | | | | |
|---|---|---|---|---|
| Condition | Action | Outcome | Participant Report | Error |
| Baseline action | | None | Action | Error= perceived time - actual time |
| Baseline outcome | None | | Outcome | Error= perceived time - actual time |
| Active action | | | Action | Error= perceived time - actual time |
| Active outcome | | | Outcome | Error= perceived time - actual time |

**Table 1. Intentional binding measurement blocks and calculations for error estimation.**

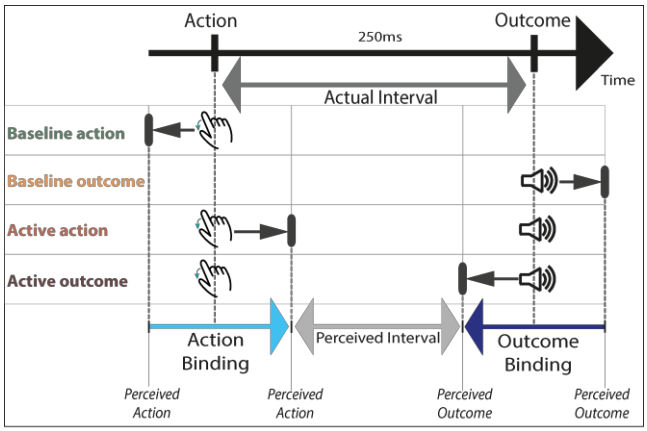



**Figure 3. Intentional binding conditions and calculation formulas. For action, participants executed either a physical button-press or a touchless button-click gesture. For outcome, they received one action confirmation: visual on-screen, auditory (a beep), vibrotactile or mid-air haptic feedback.**

As shown in Figure 3, in baseline conditions participants perceived the action earlier but the outcome later when compared to the actual time. However, in active conditions the effect was the opposite. The active conditions create an effect where action and outcome are shifted towards each other [23]. This is evidence of both predictive and postdictive influence of the origination of agency in the intentional binding paradigm [13, 43].

### The Libet clock

We employed Libet's method to measure participants' time-subjective experience, in order to investigate volitional control of movement and thus to record perception of time (the Libet clock). It consists of a clock that rotates clockwise once every 2560ms (see Figure 2 left). Participants reported the position of the clock at the moment when they either performed the action or received the outcome (see Table 1) to indicate their perceived time. The experience of agency can be also measured with alternative methods, for example "Interval estimation" [15, 43], which consists of reporting an estimate of the time interval between the action and the outcome. Another method is self-reporting questionnaires and scales that are related to binary answers about whether the user was the agent of the action or not. However, the intentional binding paradigm with the Libet Clock has been shown as a robust technique to implicitly measure the SoA [43, 45, 53, 54]. We therefore use this method in our experiments.

## TOUCHLESS BUTTON CLICK

In order to investigate the relationship between states of the in-air gesture input and the system's responses, we wanted to explore how gesture actions influence agency. We measured intentional binding during simple micro-interactions typical of desktop computing environments. We based our selection gesture on a study by Saffer, who states that "*The best, most natural designs, then, are those that match the behavior of the system to the gesture humans might actually do to enable that behavior. Simple examples include pushing a button to turn something on or off*" [55]. Consequently, we chose a fundamental gesture action to use (touchless button-click) in order to compare it with typical touch input (button-press).

In this context, a button-press movement is common in our everyday interaction with computers and smartphones. Besides, it can be reliably tracked with devices such as Leap Motion, which is specifically focused on hand and fingers tracking. In common desktop computing environments, a physical button-press generally produces three kinds of effect: (1) visual on-screen: when we press a button or key of the keyboard we normally expect a visual change on-screen (e.g. typing tasks), (2) auditory feedback: because we can perceive a click sound through mechanical pressure on the actuator; and (3) haptic: because of the obvious physical contact with the mechanoreceptors of the skin. Therefore, we provided participants with visual, auditory and haptic feedback as the outcome of our physical and gestural action input to examine how states of input (physical and touchless) map onto states of the system.

## INVESTIGATING AGENCY IN TOUCHLESS INTERFACES

Touchless systems are being used in a wide range of applications; however, the role of SoA in this kind of interaction is unknown. Does the user perceive a touchless command as being as responsive as a physical one? Does haptic feedback help to increase user's SoA in touchless interfaces? To answer these questions, we conducted two studies. In our Study 1, we explored touchless input modality and compared it with physical-based input. So far, only auditory and visual outcomes have been employed as a means of action confirmation to investigate agency (as mentioned in related work section); however, in our Study 2, we introduced haptic feedback (vibrotactile and mid-air) as a new output modality to investigate if tactile sensation can enhance users' SoA in touchless interactions.

### Method and Materials

Participants judged their perception of time by reporting the position of a rotating dot around a Libet clock at the moment when they either executed an action (baseline action and active action blocks) or received the feedback (baseline outcome and active outcome blocks) as shown in Table 1. The numbers of the clock were not used in order to avoid creating visual patterns during the task. This is because in pilot studies we noticed that participants tended to "identify" with their gaze a number as a reference, (e.g. "I'm going to do the action when the dot reaches the number 3"). This does not reflect the volition/urge to execute a motor action. Thereby participants used an external controller (Griffin Powermate USB Controller) to place the dot on the perceived position. The Libet clock size 500 pixels in diameter, was placed at the center of a screen (24 inch, 1920 x 1080 resolution). The perceived and actual times were recorded to calculate the intentional binding. In the trials with user-performed action, the action was either a touchless click gesture or a physical button-press. The outcome was presented in one of four different feedback methods: on-screen visual, auditory (a beep), wearable vibrotactile, and mid-air haptic feedback.

#### *Gesture Action*

Participants moved their index finger, mimicking a press-button action (i.e. up-down finger movement of 2 cm). The gesture was captured using a Leap Motion controller with capture rates of about 300 fps. Participants rested their hand (palm down) at a fixed position of about 20 cm height from the surface of the Leap Motion device in all feedback conditions preceding the gesture (see Figure 4). After a period of 250 ms a sensory effect was given to participants.

#### *Auditory Outcome*

Auditory stimulus is the common sensory effect used in the intentional binding paradigm. We considered audio feedback to have baseline comparison with new outcome modalities. In the conditions when there was auditory feedback, participants heard a tone that lasted 200 ms at 900 Hz in frequency using headphones. However, they always wore headphones during the full study.

#### *Visual Outcome*

Visual feedback was in the form of an on-screen button (250 pixels in diameter) that was presented at the center of the screen, and inside the Libet clock. When participants

performed the click gesture, they could see the animation of this button changing state (the button sank as if it had been pressed; changed from red to green; and returned to its original state after 200 ms). The procedure for presenting visual stimuli and the Libet clock is similar to previous studies [37, 47]. Possible time delays due to the refresh rate of the screen used in our study (60 Hz) in the visual conditions on-screen, including the rotation of the Libet clock, was compensated for by following the procedure of previous studies [58]. We executed a preliminary test with a photodetector and high-speed camera placed in the middle of the screen in order to count the number of frames shown within specific periods of time. This was done in order to identify and compensate for missing frames. Our system was consistent in missing one frame in each trial, so to correct this delay, we subtracted the duration of one tick (16.66ms) from our interval durations as in [17].

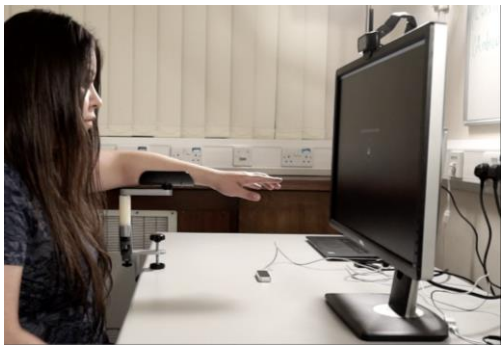


**Figure 4. Experimental setup.**

*Vibrotactile Haptic Outcome*

Vibrotactile feedback was given to participants using a wearable glove with an embedded coin vibration motor (model 310-103 by Precision Microdrives), 1cm in diameter and positioned in the glove so that the vibration is provided on a participant's fingertip (index finger). This motor vibrated at a speed of 12,000 rpm and 250 Hz in frequency. The typical rise time of 87 ms was compensated for to preserve timing as accurately as possible. Each vibration lasted 200 ms, which was easily recognizable over the tactile channel [19]. Participants did not wear the glove during visual, auditory and mid-air haptic feedback blocks.

*Mid-air Haptic Outcome*

Mid-air haptic feedback was provided using the UltraHaptics kit [8]. This device uses low-intensity and low-frequency ultrasound pressure waves to create multiple focal points in mid-air for tactile-sensation. The user can perceive the focal points using bare hands due to the receptors in the hand evoking a haptic sensation. To equalize two haptic feedback conditions in terms of stimulation area, we simulated vibrotactile outcome features with an UltraHaptics kit. Five focal points were created on participants' fingertip (index finger) to cover an area of 1 $cm^2$ with the same frequency as the vibrotactile condition (250 Hz). The stimulation lasted for 200ms.

## STUDY 1. TOUCHLESS VS PHYSICAL

In this experiment, we compared physical-based and gestural-based touchless inputs preceding auditory and visual feedback as the outcome. This resulted in four combinations of action + outcome: physical & auditory, physical & visual, gestural & auditory and gestural & visual, as shown in Figure 5.

### Procedure

Participants were asked to sit in front of a screen at a distance of about 100 cm. Every trial started when they pressed a footswitch to indicate they were ready to start. After this, a Libet clock with a rotating dot was presented at the center of a screen. The dot always started at a random position. After one full revolution of the dot, participants were asked to perform the action: a physical button-press using a keyboard (space key) or a click gesture in mid-air. For touchless action, the hand always stayed palm down and rested on top of a supporting structure (as in Figure 4). For physical action, this structure was not used and the Leap Motion device was replaced by a computer keyboard. Participants always executed the action (gestural and physical) using their dominant hand.

After a period of 250 ms, the outcome was presented in the form of auditory (a beep) and visual feedback on-screen. Then, participants judged their perception of time by reporting the position of the dot on the clock. Participants wore noise-cancelling headphones to eliminate any audible noise from the devices. Participants performed 20 trials in each condition resulting in 320 trials per participant (20 trails x 4 intentional binding blocks x 4 combinations of action + feedback). The experiment was completed in a maximum time of 90min; there was a short break between conditions. Figure 5 shows the procedure of a single trial.

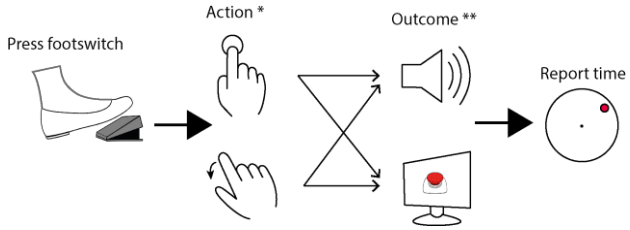



**Figure 5. Experimental trial of Study 1 (*not done in baseline outcome blocks, ** not done in baseline action blocks).**

### Participants

Twelve right-handed participants (4 Male, mean age=30.92 years, SD=3.03) took part in the experiment. They had normal or corrected-to-normal vision. The local ethics committee approved this study and participants were not paid for their participation.

### Results

A Repeated Measure design was used to compare the effects of touchless input modality with physical-based input and visual and auditory feedback. We report the

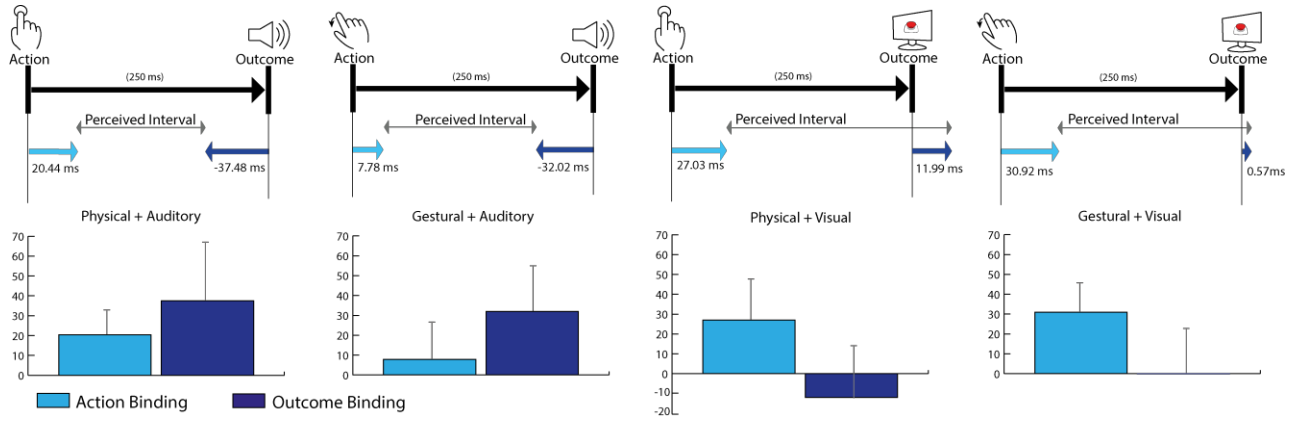



**Figure 6. Average of action binding and outcome binding in milliseconds of each action and outcome modality. The sign of outcome binding effects on the chart bars has been inverted to allow for comparison with action binding. Error bars represent standard error of mean.**

partial eta squared ($\eta_p^2$) as a measure of effect size. According to Cohen [11], a value of 0.01 is considered a small effect, 0.06 a medium effect and a value of 0.14 or greater, a large effect size.

| Action + Feedback | Action Binding | Outcome Binding | Total Binding |
|---|---|---|---|
| Physical + Auditory | 20.44 ms (43.79 ms) | -37.48ms (106.23 ms) | 57.92 ms (103.88 ms) |
| Physical + Visual | 27.03 ms (73.50 ms) | 11.99 ms (92.28 ms) | 15.04 ms (109.42 ms) |
| Gestural + Auditory | 7.78 ms (66.81 ms) | -32.02 ms (81.73 ms) | 39.80 ms (106.01 ms) |
| Gestural + Visual | 30.92 ms (52.76 ms) | 0.57 ms (81.25 ms) | 30.87 ms (68.30 ms) |

**Table 2. Average of action, outcome and total binding in milliseconds (with standard deviation in brackets) grouped by combination of action & outcome.**

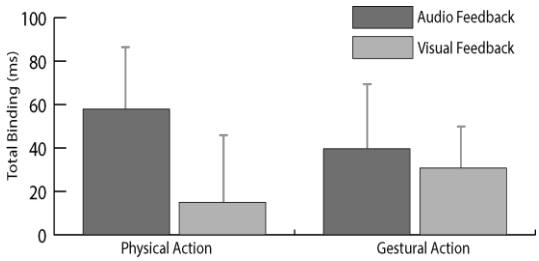



**Figure 7. Average of total binding in milliseconds for each combination of action and outcome. Error bars represent standard error of mean.**

A 2X2 within subjects' ANOVA, with the type of action (touchless gesture-based click vs physical button-press) and the type of feedback (visual vs auditory) as factors, revealed no significant effect of type of action on total binding $F_{(1,11)}$=0.003, $p$=0.96, $\eta_p^2$=0.00. We also found no significant interaction between the type of action and type of feedback $F_{(1,11)}$=0.63, $p$=0.45, $\eta_p^2$=0.05. However there was a significant main effect of the type of feedback $F_{(1,11)}$=5.31, $p$=0.04, $\eta_p^2$=0.33 with the auditory feedback scoring higher compared to the visual feedback. Figure 7 shows the average total binding with different action and feedback modalities.

An identical ANOVA was then performed for the action binding, showing no significant interaction $F_{(1,11)}$=0.36, $p$=0.56, $\eta_p^2$=0.03, and no main effect of the type of action $F_{(1,11)}$=0.12, $p$=0.74, $\eta_p^2$=0.01 and the type of feedback, $F_{(1,11)}$=0.79, $p$=0.39, $\eta_p^2$=0.07.

The outcome binding, however, showed a significant main effect of the type of feedback $F_{(1,11)}$=9.17, p=0.01, $\eta_p^2$=0.45, with auditory outcome producing an increased binding in both the physical button-press ($M$=-37.48ms, $SD$=106.23ms) and the touchless gesture-based click ($M$=-32.02ms, $SD$=81.73ms) compared to visual feedback respectively in the physical action ($M$=11.99ms, $SD$=92.28ms) and in the touchless gesture-based click ($M$=0.57ms, $SD$=81.25ms). A breakdown of these means in relation to action and outcome binding is shown in Table 2. Figure 6 shows action binding and outcome binding effects.

### Discussion of Study 1

Our results from the Study 1 revealed an intentional binding effect when both input modalities gestural and physical preceded an auditory feedback. However, this effect was not observed with visual feedback. As shown in Figure 6 the visual outcome did not shift towards the action. This suggests that the touchless system exhibited significantly more intentional binding when the input action was accompanied by auditory outcome compared with visual outcome. As expected, the physical button-press preceding an auditory outcome produced intentional binding, as shown in a large number of studies on SoA.

Interestingly we found no statistically significant difference in the action binding across the different combinations of action and outcome. This could suggest that participants may have perceived the touchless action to be as responsive as the physical action in terms of intentional binding, even when the touchless action did not involve typical characteristics of touching and object i.e. proprioceptive perception. The proprioceptive perception plays an

important role in terms of feeling an immediate haptic feedback (as in pressing a physical button) additionally from the feedback system. In the previous work from Coyle et al. [12], participants reported increased intentional binding for skin-based input modality as this action involved tactile sensation in both the finger and the arm. Thereby, this seems a challenge for touchless action where implicit tactile feedback is not committed.

However, although in our touchless condition there was not a simultaneous action-feedback like in physical button-press, interestingly we still found an intentional binding effect, as the touchless action execution always involved participants' motor movement followed a prior intention. Previous studies have suggested that the SoA principally arises due to internal motor signals [6, 42] and also that intention to act influences action attribution, when reafferent signals (e.g. motor or visual) match with intention retrospectively [9, 10, 64]. Thereby ideomotor signals produced by the touchless action could have served as a contributory factor in our results on intentional binding.

Furthermore, we also attribute these findings to the influence of the postdictive model of origination of agency. As we state, "it is not necessary to physically touch an object to have the perception of a "button-press" if it is associated with an effect in response (see Visual, audio and haptic feedback section). Although the touchless action did not involve immediate tactile feedback, participants always received a confirmation with a visual or auditory outcome. Similar accounts were reported in [3, 28, 63], where subjects reported feelings of agency even when there was no cause preceding the effect, but just the effect itself. Yet in our studies participants always had an intention to act and thereby a motor movement preceding an outcome. This could have contributed to the intentional biding effect shown in our results.

## STUDY 2. TOUCHLESS VISUAL & HAPTICS

This experiment aimed to investigate if haptic feedback can improve participants' SoA in gesture-based touchless interaction. For this, we measured intentional binding both with and without haptic feedback.

### Procedure

Participants in Study 2 used the same experimental procedure used in Study 1, with one exception. Whereas participants in Study 1 performed two kinds of actions (physical and touchless) and received two kinds of feedback (auditory and visual), in the second study participants performed only the touchless-based action and received visual, vibrotactile and mid-air haptic feedback (Figure 8). Both kinds of haptic feedback were provided on participants' dominant hand (index finger). Participants wore noise-cancelling headphones to eliminate any audible noise from the devices. Participants performed 30 trials for each condition resulting in 360 trails per participant (30 trials x 4 intentional binding blocks x 3 combinations of action + feedback). The experiment was completed in a maximum time of 90min; there was a short break between conditions. Figure 8 shows the procedure of a single trial.

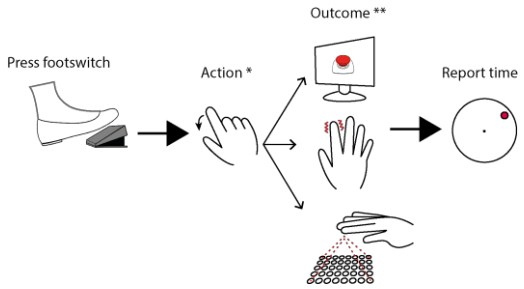



**Figure 8. Experimental trial of Study 2 (*not done in baseline outcome blocks, ** not done in baseline action blocks).**

### Participants

Twelve right-handed participants (4 Female, mean age=30.33 years, *SD*=3.86), took part in the experiment. They had normal or correct-to-normal vision. The local ethics committee approved this study and participants were not paid for their participation.

### Results

A One-way Repeated Measure ANOVA was conducted to compare the effect of the three type of feedback (visual vs vibrotactile vs mid-air haptic) on the action, outcome and total binding. Results show a significant effect on the total binding $F_{(2,22)}$=4.96, $p$=0.02, $\eta_p^2$=0.31 depending on the type of feedback. Post-hoc comparisons using Bonferroni correction showed that there is a statistically significant difference in the total binding specifically in the mid-air haptic feedback (*M*=84.21ms, *SD*=111.35ms) compared to the visual (*M*=-6.41ms, *SD*=82.98ms), $p$=0.02; but no such difference was found compared to the vibrotactile condition (*M*=40.77ms, *SD*=89.84ms), $p$=0.69. The difference between the visual condition and vibrotactile was also not significant, $p$=0.23. Figure 10 shows the average total binding with different action and feedback modalities.

We found that the action binding was not significantly affected by the type of feedback $F_{(2,22)}$=0.27, $p$=0.76, $\eta_p^2$=0.02. However, crucially the outcome binding showed a significant difference $F_{(2,22)}$=0.6.74, $p$=0.005, $\eta_p^2$=0.38. Post-hoc comparisons using Bonferroni correction showed that the outcome binding was significantly greater in the mid-air haptic condition (*M*=-64.79ms, *SD*=79.58ms) compared to the visual (*M*=12.68ms, *SD*=66.07ms) condition $p$=0.02, but there was not statistically significant difference between the mid-air haptic and the vibrotactile feedback (*M*=-29.13ms, *SD*=69.75ms), $p$=0.69ms. Additionally, we found no significant difference between the vibrotactile and the visual $p$=0.23.

These findings suggest that mid-air haptic feedback produces a strongest effect in the intentional binding values and specifically in the outcome binding compared to the other modalities. A breakdown of means in relation to

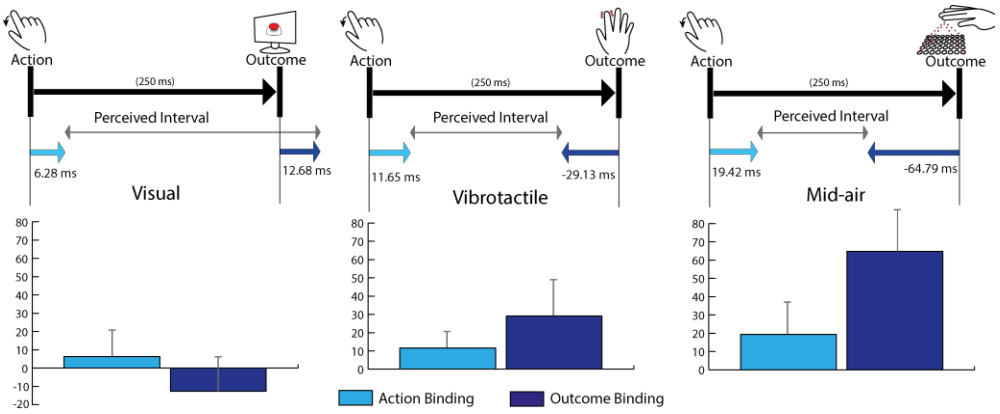



**Figure 9. Average of action binding and outcome binding in milliseconds for each feedback type (visual, vibrotactile, and mid-air). The sign of outcome binding effects on the chart bars has been inverted to allow for comparison with action binding. Error bars represent standard error of mean.**

action and outcome binding is shown in Table 3. Figure 9 shows action binding and outcome binding effects.

| Feedback | Action Binding | Outcome Binding | Total Binding |
|---|---|---|---|
| Visual | 6.28 ms (49.55 ms) | 12.68 ms (66.07 ms) | -6.41 ms (82.98 ms) |
| Vibrotactile | 11.65 ms (32.39 ms) | -29.13 ms (69.76 ms) | 40.77 ms (89.84 ms) |
| Mid-air | 19.42 ms (62.22 ms) | -64.79 ms (79.58 ms) | 84.21 ms (111.35 ms) |

**Table 3. Average of action, outcome, and total binding (with standard deviation in brackets) grouped by feedback type.**

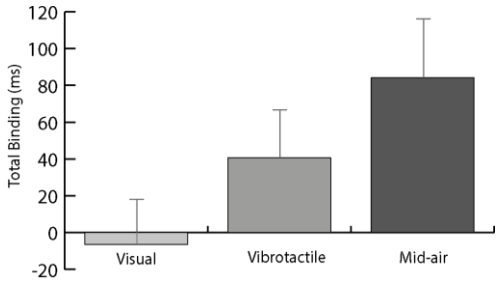



**Figure 10. Average of total binding in milliseconds for each feedback type (visual, vibrotactile, and mid-air). Error bars represent standard error of mean.**

We additionally performed further analysis using independent sample t-test to compare the effect of the intentional binding with auditory feedback in touchless modality in the Study 1 with the mid-air haptic feedback in the Study 2. Results showed no significant difference on the total binding $t(22)=0.99$, $p=0.33$ between the auditory condition of the Study 1 ($M=39.80$ms, $SD=106.02$ms), and the mid-air haptic condition ($M=84.21$ms, $SD=111.35$ms) of the Study 2. These results were also not significant for the outcome binding $t(22)=0.68$, $p=0.32$, in the auditory condition ($M=-32.02$ms, $SD=81.73$ms) compared to the mid-air haptic condition ($M=-64.79$ms, $SD=79.58$ms).

### Discussion of Study 2

Our results from the Study 2 revealed an intentional binding effect when the touchless input modality preceded a haptic feedback. However, this effect was not observed with visual feedback similar to Study 1. This suggests that the touchless system exhibited significant higher intentional binding when participants received a haptic confirmation rather than a visual confirmation. Crucially we found no statistically significant difference in action binding values across the outcome modalities.

Both haptic feedback conditions (vibrotactile and mid-air) shifted towards the touchless action. Interestingly we found no statistically significant difference for outcome binding between these two haptic conditions. We set both outcome conditions with the same characteristics as much as possible. This is because vibrotactile feedback is higher in intensity compared with ultrasound. However, by creating five focal points of ultrasound overlapping each other to cover the same area as the vibrotactile stimuli, we could equalize between these two conditions.

## GENERAL DISCUSSION

Our results revealed the existence of intentional binding effect in touchless gesture-based interactive applications. From our two studies, we found that gesture-based system exhibited significant higher intentional binding when the input action was accompanied by haptic or auditory outcomes compared with visual outcome. Our results from Study 1 showed action binding effect in both physical and touchless interactions with no statistically significant difference, possibly suggesting that that our click gesture input could be as responsive as the physical action in terms of intentional binding, even when no simultaneous action-feedback occurred like in physical touch events. We attribute this result to ideomotor signals and the postdictive influence of agency in the intentional binding paradigm, where participants always received an action confirmation with a visual, auditory or haptic outcome (in contrast to Coyle's work where only audio feedback was considered). Although we obtained different intentional binding values from the tasks involving gesture input and visual feedback in both studies, we found no statistically significant difference in this condition between Studies 1 and 2.

Our results from both studies showed different outcome binding effects depending on the type of feedback, with audio and haptic feedback producing higher intentional binding effect than visual feedback. Visual feedback on-screen produced the lowest intentional binding effect in both studies. This suggests that participants perceived higher perception of controlling the touchless interface when they received an auditory or haptic confirmation, rather than a visual confirmation. In cognitive neuroscience, a wide range of studies have employed audio feedback for studying agency, showing it to be a suitable technique to measure and produce SoA [1, 27, 43, 44]. However, in our Study 2, we also found an intentional binding effect with vibrotactile and mid-air haptic outcomes with no statistically significant difference between them. This suggests that if one cannot provide audio feedback it may be preferred from an intentional binding perspective to provide haptic feedback over visual-only feedback.

It is worth mentioning that we are aware that the UltraHaptics device produces sound because of the ultrasound waves emission. In the frequency at which it works, audible sound is generated from its speakers. To address this, participants were asked to wear noise-cancelling headphones, not only during this condition but also for the entire task (including all the conditions).

### Limitations

For the present work, we only collected quantitative measures. We employed the intentional binding paradigm as an implicit measure of the SoA following evidence that suggests that the increased intentional binding is related to higher experience of agency [14, 45]. However, previous studies have suggested that self-reports of agency and intentional binding may operate differently [53], therefore further research is need to investigate the relation between explicit judgement of agency and intentional binding for touchless interfaces. Additionally, in this work we put more attention on the impact of output modalities on agency and further studies are needed to examine the effect of proprioceptive perception on the SoA in mid-air interactions, possibly by using the haptic devices to create more natural perception of touching real objects. We mainly compared visual feedback with the other modalities in our two studies, thereby more direct comparison between audio and haptic feedback will be explored in future work.

### Application scenarios

In this work, we have shown types of interaction that significantly impact on users' SoA in order to provide solutions to improve touchless interfaces. Our results suggest that audio and haptic feedback are better to produce users' SoA compared with visual feedback. Although these kinds of feedback have been frequently used in past work (as seen in the related work section) the role of SoA have been unexplored. Here, we have validated these feedback types by implicit and quantitative metrics supporting their use to provide a better and more responsive interaction. Here we explain some possible application scenarios.

Interactions in Virtual Reality (VR) commonly rely on touchless actions; however, these systems often add haptic feedback, as they try to simulate real world settings in order to provide a realistic interaction. We have demonstrated that touch and touchless input modalities accompanied by mid-air haptic feedback improve users' intentional binding, which enables application scenarios for VR and bare-hands interactions. For example, by considering the role of agency in designing VR training simulators (e.g. flight or surgery), the designer can approximate agency effects in users that are similar to those in a real-life situation. In this way, their commitment to the interaction (action inputs and system responses) might be stronger, enabling better training for the professional.

It is known that audio and haptic feedback releases the visual channel to focus on additional tasks; this interplay is suitable for driving scenarios, for example. Our results showed that audio and mid-air haptic feedback improve users' SoA. This suggests that these kinds of feedback not only will help to focus driving attention but also produce the user's feeling of being in control during touchless interaction (e.g. controllers for car dashboards). Additionally, mid-air haptic feedback represents a good means for private communication in cases where audio cannot be played, allowing the user to still experience agency. By considering the SoA in interface design, we can explore a wide range of interaction paradigms that enable users' feeling of control in order create interactions that are more realistic and thus develop more responsive systems.

## CONCLUSION

Despite touchless systems being used in a wide range of applications, the role of agency in these systems had been unexplored to date. The lack of understanding this aspect, constrains the relevance of perceiving a responsive interface. In this paper, we have demonstrated by implicit and quantitative measures that touchless input could be perceived as responsive as a physical input action. Although our work focused only on a basic activation gesture (in-air button press), this creates an opportunity to offer possible solutions for designers in order to improve gesture-based touchless interfaces. Our work also suggests that audio and haptic feedback in gesture-based touchless interactions are a good candidate for increasing users' sense of being in control and feeling of interacting with a more responsive system. These findings contribute to a new area for HCI researchers to explore agency consideration in HCI design.

## ACKNOWLEDGEMENTS

We thank Luis Veloso for the video. This work has been supported by the European commission through European Research Council's starting grant (#278576) and the Mexican National Council of Science and Technology.

## REFERENCES


1. Henk Aarts, Erik Bijleveld, Ruud Custers, Myrthel Dogge, Merel Deelder, Dennis Schutter, and Neeltje EM van Haren. 2012. Positive priming and intentional binding: Eye-blink rate predicts reward information effects on the sense of agency. Social Neuroscience 7, 1: 105-112. DOI: **http://dx.doi.org/10.1080/17470919.2011.590602**
2. Steven Ashley. 2014. Touch-less control coming cars. SAE, automotive engineering: 20-23 from **http://www.visteon.com/media/touchless.pdf**
3. Domna Banakou and Mel Slater. 2014. Body ownership causes illusory self-attribution of speaking and influences subsequent real speaking. Proceedings of the National Academy of Sciences 111, 49: 17678-17683. DOI: **http://dx.doi.org/10.1073/pnas.1414936111**
4. Laura Bartoli, Franca Garzotto, Mirko Gelsomini, Luigi Oliveto, and Matteo Valoriani. 2014. Designing and evaluating touchless playful interaction for ASD children. ACM, Proceedings of the 2014 conference on Interaction design and children: 17-26. DOI: **http://dx.doi.org/10.1145/2593968.2593976**
5. Thomas Baudel and Michel Beaudouin-Lafon. 1993. Charade: remote control of objects using free-hand gestures. Communications of the ACM 36, 7: 28-35. DOI: **http://dx.doi.org/10.1145/159544.159562**.
6. Sarah-Jayne Blakemore, Daniel M Wolpert, and Christopher D Frith. 2002. Abnormalities in the awareness of action. Trends in cognitive sciences 6, 6: 237-242. DOI: **http://dx.doi.org/10.1016/S1364-6613(02)01907-1**
7. Claude Cadoz. 1994. Les réalités virtuelles.
8. Tom Carter, Sue Ann Seah, Benjamin Long, Bruce Drinkwater, and Sriram Subramanian. 2013. UltraHaptics: multi-point mid-air haptic feedback for touch surfaces. ACM, Proceedings of the 26th annual ACM symposium on User interface software and technology: 505-514. DOI: **http://dx.doi.org/10.1145/2501988.2502018**
9. Valerian Chambon and Patrick Haggard. 2013. Premotor or Ideomotor: How Does the Experience of Action Come About? Action science: Foundations of an emerging discipline: 359. DOI: **http://dx.doi.org/10.7551/mitpress/9780262018555.003.0014**
10. Valerian Chambon and Patrick Haggard. 2012. Sense of control depends on fluency of action selection, not motor performance. Cognition 125, 3: 441-451. DOI: **http://dx.doi.org/10.1016/j.cognition.2012.07.011**
11. Jacob Cohen. 1977. Statistical power analysis for the behavioral sciences (revised ed.). New York: Academic Press.
12. David Coyle, James Moore, Per Ola Kristensson, Paul Fletcher, and Alan Blackwell. 2012. I did that! Measuring users' experience of agency in their own actions. ACM, Proceedings of the SIGCHI Conference on Human Factors in Computing Systems: 2025-2034. DOI: **http://dx.doi.org/10.1145/2207676.2208350**
13. Nicole David, Albert Newen, and Kai Vogeley. 2008. The "sense of agency" and its underlying cognitive and neural mechanisms. Consciousness and cognition 17, 2: 523-534. DOI: **http://dx.doi.org/10.1016/j.concog.2008.03.004**
14. Jeffrey P Ebert and Daniel M Wegner. 2010. Time warp: Authorship shapes the perceived timing of actions and events. Consciousness and cognition 19, 1: 481-489. DOI: **http://dx.doi.org/10.1016/j.concog.2009.10.002**
15. Kai Engbert, Andreas Wohlschläger, and Patrick Haggard. 2008. Who is causing what? The sense of agency is relational and efferent-triggered. Cognition 107, 2: 693-704. DOI: **http://dx.doi.org/10.1016/j.cognition.2007.07.021**
16. Euan Freeman, Stephen Brewster, and Vuokko Lantz. 2016. Do That, There: An Interaction Technique for Addressing In-Air Gesture Systems. ACM, Proceedings of the 2016 CHI Conference on Human Factors in Computing Systems. : 2319-2331. DOI: **http://dx.doi.org/10.1145/2858036.2858308**
17. Pablo Garaizar, Miguel A Vadillo, Diego López-de-Ipiña, and Helena Matute. 2014. Measuring software timing errors in the presentation of visual stimuli in cognitive neuroscience experiments. PloS one 9, 1: e85108. DOI: **http://dx.doi.org/10.1371/journal.pone.0085108**
18. Nicolas Georgieff and Marc Jeannerod. 1998. Beyond consciousness of external reality: a "who" system for consciousness of action and self-consciousness. Consciousness and cognition 7, 3: 465-477. DOI: **http://dx.doi.org/10.1006/ccog.1998.0367**
19. George A Gescheider, John H Wright, and Ronald T Verrillo. 2010. Information-processing channels in the tactile sensory system: A psychophysical and physiological analysis. Psychology Press: 136.
20. Ivan Golod, Felix Heidrich, Christian Möllering, and Martina Ziefle. 2013. Design principles of hand gesture interfaces for microinteractions. ACM, Proceedings of the 6th International Conference on Designing Pleasurable Products and Interfaces: 11-20. DOI: **http://dx.doi.org/10.1145/2513506.2513508**
21. Sidhant Gupta, Dan Morris, Shwetak N Patel, and Desney Tan. 2013. Airwave: Non-contact haptic feedback using air vortex rings. ACM, Proceedings of the 2013 ACM international joint conference on

Pervasive and ubiquitous computing: 419-428. DOI: **http://dx.doi.org/10.1145/2493432.2493463**

22. Patrick Haggard and Manos Tsakiris. 2009. The experience of agency feelings, judgments, and responsibility. Current Directions in Psychological Science 18, 4: 242-246. DOI: **https://doi.org/10.1111/j.1467-8721.2009.01644.x**
23. Patrick Haggard, Sam Clark, and Jeri Kalogeras. 2002. Voluntary action and conscious awareness. Nature neuroscience 5, 4: 382-385. DOI: **http://dx.doi.org/10.1038/nn827**
24. Chris Harrison, Desney Tan, and Dan Morris. 2010. Skinput: appropriating the body as an input surface. ACM, Proceedings of the SIGCHI Conference on Human Factors in Computing Systems: 453-462. DOI: **http://dx.doi.org/10.1145/1753326.1753394**
25. Marc Jeannerod. 1999. The 25th Bartlett Lecture: To act or not to act: Perspectives on the representation of actions. The Quarterly Journal of Experimental Psychology A: Human Experimental Psychology 52, 1: 1-29 from **http://www.tandfonline.com/doi/abs/10.1080/713755803**
26. Petter Johansson, Lars Hall, Sverker Sikström, and Andreas Olsson. 2005. Failure to detect mismatches between intention and outcome in a simple decision task. Science 310, 5745: 116-119. DOI: **http://dx.doi.org/10.1126/science.1111709**
27. Nima Khalighinejad and Patrick Haggard. 2015. Modulating human sense of agency with non-invasive brain stimulation. Cortex 69: 93-103. DOI: **http://dx.doi.org/10.1016/j.cortex.2015.04.015**
28. Elena Kokkinara, Konstantina Kilteni, Kristopher J Blom, and Mel Slater. 2016. First Person Perspective of Seated Participants Over a Walking Virtual Body Leads to Illusory Agency Over the Walking. Scientific Reports 6. DOI: **http://dx.doi.org/10.1038/srep28879**
29. Thomas Kopinski, Stefan Geisler, and Uwe Handmann. 2013. Contactless Interaction for Automotive Applications. Mensch & Computer Workshopband: 87-94 from **http://dl.mensch-und-computer.de/handle/123456789/3368**
30. Benjamin Libet, Curtis A Gleason, Elwood W Wright, and Dennis K Pearl. 1983. Time of conscious intention to act in relation to onset of cerebral activity (readiness-potential). Brain 106, 3: 623-642. DOI: **https://doi.org/10.1093/brain/106.3.623**
31. Jaime Lien, Nicholas Gillian, M Emre Karagozler, Patrick Amihood, Carsten Schwesig, Erik Olson, Hakim Raja, and Ivan Poupyrev. 2016. Soli: ubiquitous gesture sensing with millimeter wave radar. ACM Transactions on Graphics (TOG) 35, 4: 142. DOI: **http://dx.doi.org/10.1145/2897824.2925953**
32. Hannah Limerick, James W Moore, and David Coyle. 2015. Empirical evidence for a diminished sense of agency in speech interfaces. ACM, Proceedings of the 33rd Annual ACM Conference on Human Factors in Computing Systems: 3967-3970. DOI: **http://dx.doi.org/10.1145/2702123.2702379**
33. Mingyu Liu, Mathieu Nancel, and Daniel Vogel. 2015. Gunslinger: Subtle Arms-down Mid-air Interaction. ACM, Proceedings of the 28th Annual ACM Symposium on User Interface Software & Technology: 63-71. DOI: **http://dx.doi.org/10.1145/2807442.2807489**
34. Takaki Maeda, Motoichiro Kato, Taro Muramatsu, Satoru Iwashita, Masaru Mimura, and Haruo Kashima. 2012. Aberrant sense of agency in patients with schizophrenia: Forward and backward over-attribution of temporal causality during intentional action. Psychiatry research 198, 1: 1-6. DOI: **http://dx.doi.org/10.1016/j.psychres.2011.10.021**
35. Yasutoshi Makino, Yoshikazu Furuyama, Seki Inoue, and Hiroyuki Shinoda. 2016. HaptoClone (Haptic-Optical Clone) for Mutual Tele-Environment by Real-time 3D Image Transfer with Midair Force Feedback. ACM, Proceedings of the 2016 CHI Conference on Human Factors in Computing Systems: 1980-1990. DOI: **http://dx.doi.org/10.1145/2858036.2858481**
36. Anders Markussen, Mikkel Rønne Jakobsen, and Kasper Hornbæk. 2014. Vulture: a mid-air word-gesture keyboard. ACM, Proceedings of the SIGCHI Conference on Human Factors in Computing Systems: 1073-1082. DOI: **http://dx.doi.org/10.1145/2556288.2556964**
37. John E McEneaney. 2013. Agency effects in human–computer interaction. International Journal of Human-Computer Interaction 29, 12: 798-813. DOI: **http://dx.doi.org/10.1080/10447318.2013.777826**
38. Jess McIntosh, Charlie McNeill, Mike Fraser, Frederic Kerber, Markus Löchtefeld, and Antonio Krüger. 2016. EMPress: Practical Hand Gesture Classification with Wrist-Mounted EMG and Pressure Sensing. ACM, Proceedings of the 2016 CHI Conference on Human Factors in Computing Systems: 2332-2342. DOI: **http://dx.doi.org/10.1145/2858036.2858093**
39. Clive S Mellor. 1970. First rank symptoms of schizophrenia: I. The frequency in schizophrenics on admission to hospital. II. Differences between individual first rank symptoms. The British Journal of Psychiatry 117(536): 15-23 from **http://bjp.rcpsych.org/content/117/536/15.full-text.pdf+html**
40. David Holz Michael Buckwald. 2010. Leap Motion, Inc. **www.leapmotion.com**
41. Yasuaki Monnai, Keisuke Hasegawa, Masahiro Fujiwara, Kazuma Yoshino, Seki Inoue, and Hiroyuki

Shinoda. 2014. HaptoMime: mid-air haptic interaction with a floating virtual screen. ACM, Proceedings of the 27th annual ACM symposium on User interface software and technology: 663-667. DOI: **http://dx.doi.org/10.1145/2642918.2647407**

42. James Moore and Patrick Haggard. 2008. Awareness of action: Inference and prediction. Consciousness and cognition 17, 1: 136-144. DOI: **http://dx.doi.org/10.1016/j.concog.2006.12.004**
43. James W Moore and Sukhvinder S Obhi. 2012. Intentional binding and the sense of agency: a review. Consciousness and cognition 21, 1: 546-561. DOI: **http://dx.doi.org/10.1016/j.concog.2011.12.002**
44. James W Moore, Daniel M Wegner, and Patrick Haggard. 2009. Modulating the sense of agency with external cues. Consciousness and cognition 18, 4: 1056-1064. DOI: **http://dx.doi.org/10.1016/j.concog.2009.05.004**
45. James W Moore and Patrick Haggard. 2010. Intentional binding and higher order agency experience. Consciousness and cognition 19, 1: 490-491. DOI: **http://dx.doi.org/10.1016/j.concog.2009.11.007**
46. James W Moore, Susanne A Schneider, Petra Schwingenschuh, Giovanna Moretto, Kailash P Bhatia, and Patrick Haggard. 2010. Dopaminergic medication boosts action–effect binding in Parkinson's disease. Neuropsychologia 48, 4: 1125-1132. DOI: **http://dx.doi.org/10.1016/j.neuropsychologia.2009.12.014**
47. Giovanna Moretto, Eamonn Walsh, and Patrick Haggard. 2011. Experience of agency and sense of responsibility. Consciousness and cognition 20, 4: 1847-1854. DOI: **http://dx.doi.org/10.1016/j.concog.2011.08.014**
48. Jörg Müller, Matthias Geier, Christina Dicke, and Sascha Spors. 2014. The boomRoom: mid-air direct interaction with virtual sound sources. ACM, Proceedings of the SIGCHI Conference on Human Factors in Computing Systems: 247-256. DOI: **http://dx.doi.org/10.1145/2556288.2557000**
49. Rajalakshmi Nandakumar, Vikram Iyer, Desney Tan, and Shyamnath Gollakota. 2016. FingerIO: Using Active Sonar for Fine-Grained Finger Tracking. ACM, Proceedings of the 2016 CHI Conference on Human Factors in Computing Systems: 1515-1525. DOI: **http://dx.doi.org/10.1145/2858036.2858580**
50. Rachel Nuwer. 2013. Armband adds a twitch to gesture control. New Scientist 217, 2906: 21. DOI: **http://dx.doi.org/10.1016/S0262-4079(13)60542-4**
51. Kenton O'Hara, Gerardo Gonzalez, Abigail Sellen, Graeme Penney, Andreas Varnavas, Helena Mentis, Antonio Criminisi, Robert Corish, Mark Rouncefield, and Neville Dastur. 2014. Touchless interaction in surgery. Communications of the ACM 57, 1: 70-77. DOI: **http://dx.doi.org/10.1145/2541883.2541899**
52. Sukhvinder Obhi and Patrick Haggard. 2004. Free Will and Free Won't Motor activity in the brain precedes our awareness of the intention to move, so how is it that we perceive control? American Scientist 92, 4: 358-365. DOI: **http://dx.doi.org/10.1511/2004.4.358**
53. Sukhvinder S Obhi and Preston Hall. 2011. Sense of agency and intentional binding in joint action. Experimental brain research 211, 3-4: 655-662. DOI: **http://dx.doi.org/10.1007/s00221-011-2675-2**
54. Sukhvinder S Obhi and Preston Hall. 2011. Sense of agency in joint action: Influence of human and computer co-actors. Experimental brain research 211, 3-4: 663-670. DOI: **http://dx.doi.org/10.1007/s00221-011-2662-7**
55. Dan Saffer. 2008. Designing gestural interfaces: Touchscreens and interactive devices. " O'Reilly Media, Inc.": 272.
56. Ben Shneiderman. 2005. Shneiderman's eight golden rules of interface design. Retrieved July 25: 2009 from **https://www.cs.umd.edu/users/ben/goldenrules.html**
57. Rajinder Sodhi, Ivan Poupyrev, Matthew Glisson, and Ali Israr. 2013. AIREAL: interactive tactile experiences in free air. ACM Transactions on Graphics (TOG) 32, 4: 134. DOI: **http://dx.doi.org/10.1145/2461912.2462007**
58. Neil Stewart. 2006. Millisecond accuracy video display using OpenGL under Linux. Behavior research methods 38, 1: 142-145. DOI: **http://dx.doi.org/10.3758/BF03192759**
59. Matthis Synofzik, Gottfried Vosgerau, and Martin Voss. 2013. The experience of agency: an interplay between prediction and postdiction. Frontiers in Psychology 4, 127. DOI: **http://dx.doi.org/10.3389/fpsyg.2013.00127**
60. Keisuke Takahata, Hidehiko Takahashi, Takaki Maeda, Satoshi Umeda, Tetsuya Suhara, Masaru Mimura, and Motoichiro Kato. 2012. It's not my fault: Postdictive modulation of intentional binding by monetary gains and losses. PloS one 7, 12: e53421. DOI: **http://dx.doi.org/10.1371/journal.pone.0053421**
61. Jonathan Taylor, Lucas Bordeaux, Thomas Cashman, Bob Corish, Cem Keskin, Toby Sharp, Eduardo Soto, David Sweeney, Julien Valentin, and Benjamin Luff. 2016. Efficient and precise interactive hand tracking through joint, continuous optimization of pose and correspondences. ACM Transactions on Graphics (TOG) 35, 4: 143. DOI: **http://dx.doi.org/10.1145/2897824.2925965**
62. Daniel M Wegner. 2003. The mind's best trick: how we experience conscious will. Trends in cognitive sciences

7, 2: 65-69. DOI:
**http://dx.doi.org/10.1016/S13646613(03)00002-0**

63. Daniel M Wegner, Betsy Sparrow, and Lea Winerman. 2004. Vicarious agency: experiencing control over the movements of others. Journal of personality and social psychology 86, 6: 838. DOI:
**http://dx.doi.org/10.1037/0022-3514.86.6.838**

64. Dorit Wenke, Stephen M Fleming, and Patrick Haggard. 2010. Subliminal priming of actions influences sense of control over effects of action. Cognition 115, 1: 26-38. DOI:
**http://dx.doi.org/10.1016/j.cognition.2009.10.016**

65. Daniel Wigdor and Dennis Wixon. 2011. Brave NUI world: designing natural user interfaces for touch and gesture. Elsevier, 36. DOI:
**http://dx.doi.org/10.1145/2047414.2047439**

66. Wenjie Wu and Stefan Rank. 2015. Spatial Audio Feedback for Hand Gestures in Games. ACM, Proceedings of the 3rd ACM Symposium on Spatial User Interaction: 135-135. DOI:
**http://dx.doi.org/10.1145/2788940.2794363**

67. Wenjie Wu and Stefan Rank. 2015. Responsive Environmental Diegetic Audio Feedback for Hand Gestures in Audio-Only Games. ACM, Proceedings of the 2015 Annual Symposium on Computer-Human Interaction in Play: 739-744. DOI:
**http://dx.doi.org/10.1145/2793107.2810336**

68. Zhengyou Zhang. 2012. Microsoft kinect sensor and its effect. IEEE multimedia 19, 2: 4-10. DOI:
**http://dx.doi.org/10.1109/MMUL.2012.24**