NOTE TO USERS

The Role of Expectancy and Abstinence in the Neural Response to Smoking Cues in Cigarette Smokers: an fMRI Study

Dharma T. McBride

Department of Neurology and Neurosurgery
McGill University, Montreal

Submitted February, 2005

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Master's of Science

Your file Votre référence
ISBN: 0-494-12503-9
Our file Notre référence
ISBN: 0-494-12503-9

TABLE OF CONTENTS

ABSTRACT

Recent studies using brain imaging have begun to reveal the neural systems that underlie the response to drug cues in human addicts, however, little is known about how they are affected by perceived drug availability and degree of abstinence in the user. Using functional magnetic resonance imaging (fMRI) and a cue reactivity paradigm in cigarette smokers, we have investigated, for the first time, the neural correlates of expectancy and abstinence on the drug user's response to stimuli associated with drug use. Exposure to smoking cues induced a subjective craving response and elicited activation of a distributed neural system involved in the cognitive and emotional processing of salient stimuli. We showed that in a state of anticipation to smoke, this neural response was heightened, notably in the prefrontal cortex, whereas it was absent during a state of non-expectancy. In addition, we showed that this cue-induced response was disrupted during acute withdrawal from smoking.

RESUMÉ

Récemment des études d'imagerie cérébrales ont permis d'identifier, chez le sujet toxicodépendant, les substrats neurobiologiques impliqués dans la réponse aux stimuli préalablement associés à la prise de drogues (stimuli d'appétence); toutefois nous ne savons pas si ces substrats sont modulés par le degré de sevrage et l'éventuelle accessibilité de la drogue. Nous avons utilisé l'imagerie par résonance magnétique fonctionnelle (IRMf) et un paradigme de réactivité à des stimuli d'appétence (séquences vidéo de fumeurs), afin d'étudier, chez le fumeur, l'impact de l'anticipation et du sevrage sur la réponse neurobiologique d'appétence. La présentation des séquences vidéo de fumeurs augmenta la réponse subjective d'appétence (*craving*) toute en sollicitant l'activité d'une circuiterie neuronale que l'on croit impliquée dans le contrôle des processus émotionnels et motivationnels associées aux stimuli saillants. Nos données montrent que l'anticipation de fumer potentialise la réponse neurobiologique d'appétence, notamment dans le cortex préfrontal; inversement cette réponse est absente lorsque le sujet anticipait de ne pas fumer. De plus, nos résultats révèlent que le sevrage cancelle la réponse neurobiologique aux stimuli d'appétence.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor for this project, Dr. Alain Dagher, for accepting me into his lab and allowing me the opportunity to work in such a stimulating and fascinating field. I am very appreciative of his keen guidance and willingness to assist me whenever it was necessary. I am especially grateful for his generous allowance of time off, which has helped me to keep from being a dull boy.

A special thanks to Mike Ferreira and Bojana Stefanovitch for all of their help and expertise in setting up my protocol and analysis of the data. Their incredible patience and ability to handle the inevitable stress of brain imaging studies has made this undertaking as smooth as possible. Thank you to Andrew Aw for your very timely help with the analysis (and prompt updates!). I would also like to express thanks to the administrative staff (especially Monique Ledermann and Sacha Young) and research technicians of the MNI for running such a good ship.

To Isabelle Boileau, for your support, wisdom and incredible generosity of spirit, I shall never forget. And thank you also for occasionally switching the topic from neuroscience to climbing. And to Johanna Okker, for giving me the inspiration and courage to choose my path, I will be forever grateful.

Last, but not least, I would like to express my immense love and gratitude to all of my friends and family (you too, Japhy!), for all the support, understanding and love you have given me during this journey.

CONTRIBUTION OF AUTHORS

I have chosen to prepare a manuscript-based thesis, as set out in the "Guidelines Concerning Thesis Preparation". The following people are listed as co-authors on the included manuscript:

Dharma T. McBride – Performed bulk of project, including design of experiment, writing of ethics proposal, recruitment of subjects, running of experiments, analysis of data (with exceptions, see below), and interpretation of results

Andrew Aw – Performed connectivity analysis

Sean P. Barrett – Performed study which validated video stimuli

Jared T. Kelly – Performed study which validated video stimuli

Dr. Robert O. Pihl – Supervisor for Sean P Barrett and Jared T. Kelly

Dr. Alain Dagher – Supervised project, assisted with correlation analysis, helped interpret results, edited thesis

LIST OF ABBREVIATIONS

ABS – abstinent session

ACC – anterior cingulated cortex

ANOVA – analysis of variance

BA – Brodmann's area

BOLD – blood oxygen level dependent

CO – carbon monoxide

CR – conditioned response

CS – conditioned stimulus

DA – dopamine

DL-PFC – dorsolateral prefrontal cortex

DSM-IV – Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition)

EPI – echoplanar imaging

EXP – expectant group

fMRI – functional magnetic resonance imaging

mPFC – medial prefrontal cortex

MTL – medial temporal lobe

N – neutral videos

nAcc – nucleus accumbens

NON-ABS – non-abstinent session

NON-EXP – non-expectant group

OFC – orbitofrontal cortex

PCC – posterior cingulate cortex

PET – positron emission tomography

PFC – prefrontal cortex

POMS – Profile of Mood States

ppm – particles per million

PR – progressive ratio

rCBF – regional cerebral blood flow

rTMS – repetitive transcranial magnetic stimulation

QSU – Questionnaire of Smoking Urges

S – smoking videos

SD – standard deviation

SE – standard error

TE – echo time

TPQ – Tri-Dimensional Personality Questionnaire

TR – relaxation time

VP – ventral pallidum

VTA – ventral tegmental area

INTRODUCTION

1 Rationale

Tobacco usage is the single largest preventable cause of mortality in the Western world, accounting for 20% of all premature deaths (Balfour, 2002). Although studies show that 70% of smokers report a desire to quit (Benowitz, 1999), it has been shown that only about 20% of those who attempt to quit are successful for more than a year (Balfour et al., 2000). The addictive potential of tobacco lies in its principal psychoactive component, nicotine, which promotes its use despite the harmful consequences (Balfour, 2002). Like other drugs of abuse, nicotine dependence is associated with craving, which cigarette smokers claim is a major factor for both their continuation to smoke and their inability to quit (Altman et al., 1996). Of particular interest is the observation that exposure to drug-associated stimuli (e.g. sight of a cigarette, smell of tobacco smoke) is a powerful inducer of craving and drug use in addicted individuals. This same effect has been employed experimentally to investigate various features involved in the cycle of addiction. For example, use of the cue reactivity paradigm has revealed the important influence of two such factors on the course of drug addiction: the degree of abstinence and perceived drug availability. However, the impact of these factors on the underlying neural mechanisms of the response to drug cues remain poorly understood, and such a focus may prove important in the understanding and eventual treatment of addiction.

Although work with animal models has provided many advances in the understanding of the neurobiology of cue-induced drug use, the complexity of addictive behaviour in humans requires research techniques that examine dependence directly in human subjects. Therefore, the use of non-invasive brain imaging techniques to

investigate the neural circuitry of nicotine addiction in cigarette smokers is an ideal approach to this problem.

2 Objectives

The objective of this experiment was to use functional magnetic resonance imaging (fMRI) and a validated cue-reactivity paradigm to study the neural correlates of cue-induced nicotine craving in cigarette smokers. We manipulated the degree of expectancy to smoke and the degree of abstinence so as to determine the effects of these factors on the neural and subjective responses to conditioned cues.

3 Hypothesis

We hypothesized that in cigarette smokers, subjective self-reports of craving would increase in response to smoking stimuli when compared to neutral stimuli. Furthermore, we hypothesized that a state of expectancy to smoke and a state of non-abstinence would result in reports of positive, appetitive craving whereas more negative, withdrawal-mediated craving would be reported during abstinence and in a state of non-expectancy. Regarding the neural response to smoking stimuli, we proposed that exposure to smoking images (relative to neutral images) would lead to activity-related blood-oxygen-level-dependent (BOLD) signal increases in limbic and frontal regions, reflecting a neural circuitry that responds to the conditioned motivational and emotional aspects of drug cues. We hypothesized that these activations would correlate with subjective reports of craving and that this neural response to cues would be preferentially

increased during expectancy to smoke, and disrupted during a state of acute abstinence from smoking.

REVIEW OF LITERATURE

1 Neurobiology of Nicotine Addiction

It has been well established from animal studies that nicotine is the primary psychoactive and addictive component of tobacco (Stolerman and Shoaib, 1991). It has been shown that when given intravenously in rodents, nicotine will support intracranial self-administration (Schaefer and Michael, 1986), promote conditioned-place preference (Vastola et al., 2002), and increase locomotor activity (Schoffelmeer, 2002), similar to other drugs of abuse such as the psychostimulants cocaine and amphetamine. Nicotine's actions on various cortical and subcortical regions of the brain are mediated by its binding to nicotinic acetylcholine receptors, thus modulating the release of neurotransmitter from dopaminergic, cholinergic, glutamatergic, serotonergic, and GABAergic nerve terminals (Alkondon, 2000; McGehee et al., 1995; Vidal and Changeux, 1993); however, it is its facilitation of dopamine (DA) release that is believed to be primarily responsible for its addictive potential (Balfour, 2002). Indeed, a common denominator shared by most drugs of abuse, including the opiates, alcohol, psychostimulants, and nicotine, is their ability to induce DA release in the nucleus accumbens (nAcc; Di Chiara and Imperato, 1988), an area involved in reward and motivation. Nisell et al. (1994) used *in vivo* microdialysis techniques in rats to demonstrate that by infusing nicotine into the midbrain ventral tegmental area (VTA, origin of DA neurons to the nAcc), dopamine release in the nAcc could be reliably

induced. Furthermore, evidence shows that by blocking either nicotine receptors in the VTA or dopamine receptors in the nAcc, the reinforcing properties of nicotine can be attenuated (Hildebrand et al., 1998).

2 Drug Craving

Craving is defined here as the desire for the previously experienced effects of a substance, such as those produced by nicotine. The importance in studying craving as a construct in drug addiction stems mainly from its common reference by drug addicts, who claim that it is a prime motivator for drug use (Anton, 1999). Classical conceptualizations of drug dependence have generally provided two main frameworks to describe the concept of craving: positive and negative reinforcement models.

2.1 Positive Reinforcement Models of Craving

Positive reinforcement models of drug abuse centre on the ability of drugs to act as incentives (positive reinforcers), and so maintain drug-seeking behaviour through their mental representation as rewards (Stewart et al., 1984). Within this framework, positive, or appetitive, craving is a conditioned emotional response characterized by the anticipation of the positive subjective state that has come to be associated with drug use (i.e. euphoria, elevated alertness, etc.). A main critique of this theory lies in a proposed dissociation between pleasure and drug use. According to the incentive-sensitization model of drug abuse (Robinson and Berridge, 1993), the progression from casual drug use to a state of drug dependence is paralleled by a shift in the user from drug-liking to drug-wanting. As such, they propose that tolerance develops to the pleasure associated with drug-taking, and thus the anticipation of these effects (i.e. positive craving) should

only be temporary. However, this view remains controversial in light of evidence from an animal study which shows that the increase in the motivational aspects of cocaine intake in rats over time coexists with an increase in the drug's rewarding effects (i.e. in the absence of tolerance; Deroche et al., 1999). Nonetheless, pharmacological intervention has been shown to be capable of decreasing the subjective effects of drugs, while not affecting either the urge for, or consumption of, the drug (Haney et al., 1998). Therefore, it would seem that positive craving cannot fully explain drug-seeking behaviour.

2.2 Negative Reinforcement Models of Craving

In contrast to the positive, incentive driven model, negative reinforcement models of craving state that the primary motivation to take drugs is to prevent withdrawal symptoms. Negative craving is characterized by the desire to take a drug in order to relieve the aversive symptoms of withdrawal and is thought to reflect an unconditioned response to removal of the drug from the body (Koob and LeMoal, 1997). Nicotine withdrawal symptoms are both affective (i.e. anxiety, dysphoria, irritability, inability to concentrate) and somatic (i.e. sweating, tremors, weight gain) in nature, though clinical observations assert that the affective signs are more relevant to craving and present more of an obstacle to quitting (Markou et al., 1993). However, there exists substantial evidence contrary to this model, including the observation that craving often persists long after withdrawal symptoms have ceased (e.g. weeks for nicotine; Miyata and Yanagita, 2001), that some highly addictive drugs (e.g. psychostimulants) do not exhibit strong withdrawal symptoms (West and Gossop, 1994), and that craving can actually be induced by administration of the drug itself (i.e. in the absence of any withdrawal; Shaham et al.,

2003). As such, factors other than the desire to relieve withdrawal symptoms must exist in order to explain drug use.

2.3 Multifactorial Approach to Craving

In response to critique for both the positive and negative reinforcement models of craving, a multifactorial approach was conceived so as to incorporate both theories into a unified model (Baker, 1987). In this framework, both models can coexist, though it is hypothesized that depending on contextual state, such as degree of abstinence, one form of craving will predominate over the other. The Questionnaire of Smoking Urges (QSU; Tiffany and Drobes, 1991), which assesses both negative (Factor I) and positive (Factor II) reinforcement properties was created to test such hypotheses. Accordingly, Willner (1985) supported this model by showing that increasing abstinence from smoking could shift craving from a positive to a negative reinforcement contingency. Interestingly, the QSU was also shown to be sensitive to smoking-related cues, with a maximal effect during a non-abstinent state (Morgan et al., 1999), supporting the idea that craving during abstinence is less dependent on external cues and arises from an unconditioned homeostatic withdrawal response. This multifactorial model has provided a more realistic method to gauge craving, but remains limited by the ability to accurately measure subjective states across a wide range of contexts (Sayette et al., 2000).

2.4 Cognitive Theory of Drug Use

Despite the general acceptance that craving plays a key role in the persistence of drug use in addicts, some claim that such a link may be overstated. For example, empirical evidence suggests that drug craving is not a reliable predictor of relapse in drug addicts and that in experienced addicts drug use is commonly not preceded by craving

(Tiffany, 1999). Tiffany (1990) has proposed a cognitive processing model of drug use, in which drug use-related behaviour represents non-conscious, automatic processes that are independent of craving. In this framework, exposure to drug-associated cues or situations would be sufficient to trigger programmed drug-seeking behaviour, and craving (conscious, non-automatic processes) would only play a role when conflict exists, such as when the drug needs to be procured or if there is a conscious effort to remain abstinent. This theory would suggest that there may be more reliable indicators of drug-seeking behaviour than craving, which may be revealed by investigating the underlying neural response to drug cues.

3 Conditioned Drug Cues

3.1 Cue-reactivity Paradigm

Drug craving and use are influenced by a variety of internal and external factors, such as degree of withdrawal, perceived availability, and stress, but perhaps most importantly, exposure to conditioned cues. Using the framework of classical conditioning, drug cues (conditioned stimuli, CS) are previously neutral stimuli or contexts that have been consistently paired with drug use, and upon exposure, induce a conditioned response (CR) in the drug user. It is thought, from everyday observations, that these cues (e.g. drug paraphernalia, drug use situations) are sufficient in both maintaining use and inducing relapse in addicts (Childress et al., 1993). However, this same phenomenon can be exploited clinically in order to better understand the addiction process. As such, many experiments have employed the cue-reactivity paradigm, in which drug users are exposed to conditioned stimuli via various methods (e.g. videos,

pictures, scripts) and their reactions are measured. Such measurable responses are generally either subjective (i.e. craving) or autonomic (i.e. heart rate, galvanic skin response, skin temperature) in nature. A meta analysis of cue reactivity across multiple drugs of abuse (e.g. cocaine, alcohol, opiates and nicotine) has demonstrated that conditioned cues are reliable inducers of these responses in an experimental setting (Carter and Tiffany, 1999), however cue-induced craving seems to be more robustly generated than cue-induced autonomic responses such as heart rate and sweating. In addition to studying the nature of addiction, the cue reactivity paradigm can also be employed to test and further investigate potential treatment methods (Drummond et al., 1995). For example, the efficacy and mechanism of action of various pharmacological treatments for nicotine addiction has been studied using the cue reactivity paradigm (Le Foll et al., 2003; Brody et al., 2004).

In addition to subjective and physiological measures of cue reactivity, it is also possible to study the behavioural (i.e. drug-seeking) response that drug cues elicit. In this regard, animal models have proved useful. For example, in a model of cue-induced relapse, reinstatement of cocaine self-administration was demonstrated when withdrawn rats, trained to lever press for the drug, were re-exposed to conditioned cues (Ciccocioppo et al., 2001). Another tool that has been used in animals to investigate the behavioural elements of drug addiction is the progressive ratio (PR) schedule. In this method, animals trained to lever press for an addictive drug are required to increase their number of responses in order to obtain further infusions of the drug. Therefore, this can gauge the amount of work the animal is willing to do to receive the drug, and has provided a means to measure the cue-induced behavioural response in drug addiction.

For example, using a PR schedule, Donny et al. (1999) showed that exposure to stimuli paired with drug self-administration significantly increased the time necessary for nicotine-dependent rats to undergo extinction of lever pressing. Although similar findings using the PR task have not reproduced such results in smokers (Morgan et al., 1999), it is commonly believed that drug cues are a key inducer of relapse in cigarette smokers (Tiffany, 1990), cocaine users (Childress et al., 1988), alcoholics (Rohsenow et al., 1994), and heroin addicts (Childress et al., 1986). As such, drug cues seem to be capable of driving behaviours that both sustain drug taking and hinder attempts to abstain from drug use.

3.2 Theories of Cue Reactivity

Similar to the theories posited to explain craving, theories of the response to drug cues are based on a classical conditioning paradigm. In this regard, the two main models of cue reactivity differ mainly in the nature of the CR that the CS (i.e. drug cues) evokes in the drug user. In the incentive-appetitive model (Stewart et al., 1984), it is proposed that drug cues induce a CR that reflects the positive, incentive properties of the drug and are therefore isodirectional to the effects of the drug. In contrast, in the withdrawal-aversive model (Wikler, 1948; Siegel, 1983), drug cues induce a CR that is similar to the withdrawal state and opposite to that of the drug. Accumulated evidence seems to support the former theory. For example, a meta analysis of cue reactivity has shown that drug cues generally produce an increase in heart rate and sweat gland activity, an autonomic response that is similar to the physiological effects of many drugs (i.e. nicotine, cocaine and alcohol), thus supporting the incentive-appetitive model (Carter and Tiffany, 1999). However, this generalized CR to drug cues is counterdirectional to the

physiological effects of opiates, suggesting that the autonomic response may also be explained by general arousal, and not completely dependent on conditioning effects (Carter and Tiffany, 1999). Further research is necessary to adequately determine the mechanisms underlying the cue reactivity, as this could provide possible avenues for treatment. For example, under a classical conditioning paradigm, it is theoretically feasible to treat addiction using extinction therapy, in which the craving and drug-seeking reactions (CR) to drug cues (CS) could be removed by repeated unreinforced exposure to these stimuli (Drummond et al., 1995).

3.3 Dopamine and Cues in Drug Abuse

The mesolimbic dopamine system consists of DA neurons of the midbrain VTA that project to various limbic regions of the brain (e.g. amygdala, hippocampus, nucleus accumbens, etc.), wherein they influence widespread functions, such as memory, emotion and motivation (Spanagel and Weiss, 1999). Accumulating evidence supports the notion that this system is central to the neurobiology of drug addiction, including the behavioural response to conditioned cues. Many insights into the neural correlates of cue reactivity have come from animal studies in which exposure to drug cues promotes self-administration, often after a period of abstinence, thus simulating relapse. Interestingly, such studies have shown that this cue-induced response can be blocked by disrupting specific regions of the mesolimbic system (e.g. basolateral amygdala), either by specific lesions (Meil and See, 1997) or by infusion of dopamine receptor antagonists (Ciccocioppo et al., 2001). The role of the mesolimbic system was further elucidated in a hallmark electrophysiological study in monkeys which showed that DA neurons of the VTA fire in response to cues predictive of reward (Schultz et al., 1998). Based on these

findings, current dopamine-based theories of drug addiction (Schultz et al., 1998; Robinson and Berridge, 1993) highlight the relation between dopamine release and the perception of salient stimuli. These theories hypothesize that dopamine release acts as a learning signal that attributes environmental stimuli with incentive salience, the internal representation of an object's reward value. Although such an effect is normally reserved for biologically significant rewards such as food, water and sex, the release of dopamine by drugs of abuse causes them to be labelled as rewarding, and by association, stimuli that are paired with drug use become secondary reinforcers and can motivate drug taking. In fact, animal work has demonstrated that drug-related cue exposure is capable of increasing DA release in the nAcc, in a manner akin to the drug itself (Fontana et al., 1993). Similar evidence exists for humans, as blockade of dopaminergic activity with haloperidol has been shown to decrease cue-induced craving in cocaine users (Berger et al., 1996). According to the incentive-sensitization view of drug addiction (Robinson and Berridge, 1993), sustained DA release during chronic drug abuse causes long term neuroadaptations to brain reward systems, rendering them hypersensitive to drugs and drug cues.

4 Brain Imaging Techniques

Although the use of animal models has proved invaluable in laying down the mechanistic foundations of drug addiction, they remain limited in their face validity to the primarily human disorder. For example, the investigation of the neural correlates of subjective states such as craving and anticipation of drug use is not feasible in animal models and can only be explored using non-invasive methods in human subjects. As

such, the advent of brain imaging techniques has allowed for greater insight into the addictive process in humans. The two most commonly used brain imaging methods are functional magnetic resonance imaging (fMRI) and positron emission tomography (PET).

4.1 Functional Magnetic Resonance Imaging

Both fMRI and PET are based on the principle that a task-dependent change in neuronal activity leads to a concomitant change in cerebral blood flow (rCBF) in that region, which can be localized and measured by techniques unique to the method (Phelps, 2000; Logothetis and Wandell, 2004). It is believed that an increase in (glutamatergic) neuronal activity induces an increase in blood flow to that area, thus supplying the oxygen and glucose necessary to fulfill the metabolic demands that accompany this activity. In fMRI, it is believed that the increase in neuronal firing (and therefore blood flow) leads to a proportional increase in regional oxyhemoglobin content of the blood (Heeger and Ress, 2002; Logothetis and Wandell, 2004). A difference between the paramagnetic properties of oxyhemoglobin and deoxyhemoglobin forms the basis of the fMRI signal. As regional oxygen influx exceeds its consumption, the resultant change in blood deoxyhemoglobin produces a blood-oxygen-level-dependent (BOLD) signal that can be measured by the scanner.

Generally, brain imaging experiments consist of both a test condition (e.g. exposure to drug stimuli) and a neutral baseline condition (e.g. exposure to neutral stimuli). By measuring the difference in BOLD signal (i.e. neuronal firing) between the two cognitive states, combining the data for multiple subjects and translating the data into a standard stereotaxic space, the brain regions selectively activated by the test condition can be determined.

5 Brain Imaging of Drug Addiction

5.1 Neurobiology of Nicotine in Humans

The two most common brain imaging techniques used to study the neural systems involved in either the direct effects of drugs or their cues are PET and fMRI. Several imaging studies looking at the direct influence of nicotine in smokers have attempted to reveal the network mediating nicotine's effects on the brain (Rose et al., 2003; Stein et al., 1998; Domino et al., 2000a, 2000b). Taken together, these studies revealed a nicotine-induced increase in blood flow in the reticular formation, thalamus, nucleus accumbens and frontal regions, with decreases found in the amygdala. Interestingly, there seems to be a significant, yet not complete, degree of overlap between the brain response to the drug and the brain response to stimuli associated with the drug.

5.2 Neural Correlates of Cue-Induced Drug Craving

Although much research has been done concerning the effect of conditioned drug stimuli on the subjective and physiological aspects of drug addiction, only recently have brain imaging techniques allowed for the study of the neural correlates of cue reactivity. Numerous brain imaging studies have investigated the neurobiology of cue-induced craving for a number of drugs of abuse, including cocaine (Grant et al., 1996; Childress et al., 1999; Garavan et al., 2000; Bonson et al., 2002), alcohol (Modell et al., 1995; George et al., 2001; Tapert et al., 2003), opiates (Sell et al., 2000; Daglish et al., 2001), and nicotine (Due et al., 2002; Brody et al., 2002). These studies suggest that drug cues activate a distributed neural network involved in the processing of the motivational, cognitive, and emotional aspects of salient stimuli. In the case of cigarette smokers

exposed to smoking stimuli, two imaging studies have recently been performed. In one study using PET (Brody et al., 2002), smoking cues induced activation of the anterior cingulate (ACC) and orbitofrontal cortex (OFC), with positive correlations found between cue-induced craving and metabolism in the OFC, dorsolateral prefrontal cortex (DL-PFC) and insula. In the other study, which used fMRI (Due et al., 2002), cigarette smokers had cue-induced activation of mesolimbic (VTA, amygdala, hippocampus, dorsomedial thalamus) and visuospatial (DL-PFC, posterior parietal) pathways. As evident from these studies, inconsistencies often exist regarding the brain areas activated by conditioned cues; however this likely reflects methodological differences such as the choice of imaging modality, stimulus presentation, and data analysis methods. Despite these differences, the areas most consistently highlighted in these imaging studies of cue-induced craving consist of limbic and prefrontal cortical regions, including the OFC, ACC, DL-PFC, amygdala, and insula. In addition, neural activity in the OFC, DL-PFC, amygdala and insula was shown to positively correlate with subjective self-reports of cue-induced craving, indicating an active mesocorticolimbic system during craving. Interestingly, one cue-induced cocaine craving study (Wexler et al., 2001) revealed that activation in the ACC preceded the onset of craving and was exhibited in some subjects who did not crave. This suggests that the neural response to conditioned cues can also be independent of craving.

5.2.1 Orbitofrontal Cortex

The prefrontal cortex, which includes the OFC, ACC, and DL-PFC, shapes goal-directed behaviour by virtue of its ability to formulate a proper reaction in light of external stimuli, past experiences and current internal drive state (Miller, 2000). As it

pertains to reward-driven behaviour, the OFC plays a key role within the prefrontal cortex information-processing network. The OFC receives input from various sensory modalities, limbic and prefrontal regions, as well as dopaminergic projections from the midbrain (Rolls, 2000). This makes the OFC ideally located to regulate reward learning processes. Reward learning involves the storage and continuous updating of the motivational value of external stimuli, a process that is affected by drug taking. For example, the finding that the OFC responds preferentially to drug cues (relative to neutral cues) confirms the salience that these previously neutral stimuli have acquired through their association with drug use. Also of great interest here is the ability of the OFC to incorporate current reinforcement contingencies, such as internal drive state and perceived availability of the reinforcer, into the neural representation of reward for an object. This is illustrated by the effects of lesions to the OFC, which disrupt the ability to adapt behaviour when changing external circumstances demand it (e.g. social conventions; London et al., 2000). The OFC's ability to respond to changing internal state is demonstrated by the observation that neurons in this region will fire in response to cues predictive of food when an animal is hungry, but will stop firing upon satiation (Rolls et al., 1989). This contextual processing ability of the OFC will likely have implications for its role in expectancy and withdrawal in drug addiction.

5.2.2 Anterior Cingulate Cortex

The anterior cingulate is the most commonly reported brain region involved in imaging studies of the response to drug cues and as such, may be important to the maladaptive state of addiction. Similar to the OFC, the ACC has connections with other prefrontal areas, limbic regions and receives dopaminergic input, implying a role in

reward-driven behaviour (Paus, 2001). Many theories have been proposed to explain the function of the ACC, including roles in emotional processing (Phillips et al., 2003), conflict detection in cognitive tasks (Carter et al., 1999), and general arousal (Paus, 2001). So far, studies of drug cues have not been able to conclusively provide support for any one theory in particular, though they do demonstrate that the ACC is a critical area involved in the processing of salient stimuli. Interestingly, the ACC is also closely connected with motor areas, demonstrating its role in mediating behavioural responses (Paus, 2001). Activation of the ACC in response to drug cues could represent a crucial interface by which these stimuli induce a drug-seeking response in the addicted individual. Interestingly, it has been proposed that this cue-induced activity in the ACC may be a neural correlate of the lack of impulse control that is such a trademark of drug addiction (Goldstein and Volkow, 2001).

5.2.3 Dorsolateral Prefrontal Cortex

The DL-PFC is a key executive centre of the brain implicated in working memory, wherein goal-directed information is manipulated and monitored for subsequent use (Petrides, 2000). The DL-PFC may function to coordinate and plan various cognitive tasks necessary for the behavioural response to salient stimuli, including those associated with drug use. As it pertains to drug use, the DL-PFC may be critical in manipulating multiple sources of information related to drug-seeking behaviour, such as the drug's availability, internal drive state, and external cues associated with drug use. Consistent with its connections with the premotor cortex (Petrides and Pandya, 1999), the DL-PFC may be a key intermediate between the processing of goal-related information and the appropriate action to obtain the reward (Dehaene and Changeux, 2000). The degree of

motivation to obtain a reward may influence the DL-PFC by way of its connections with the OFC (Ongur et al., 2003). Supporting this is a recent study in monkeys showing that exposure to reward cues elicited firing of DL-PFC neurons in conjunction with neurons of the motor system, and that this response varied directly with the magnitude of the reward (Roesch and Olson, 2003). However, as the DL-PFC is a widespread area involved in various cognitive functions (Krawczyk, 2002), it is likely that it may have multiple roles in the response to conditioned drug cues. For example, in light of its role in the retrieval of episodic memory, it has been proposed that drug cue-induced activation of this area represents reactivation of memories associated with former drug use (Grant et al., 1996).

5.2.4 Amygdala

The amygdala is a region important in the emotional and motivational processing of discrete environmental stimuli, and as part of the mesolimbic system, is likely responsible for labelling drug cues with emotional significance (Meil and See, 1997). The amygdala has reciprocal connections with a wide array of brain areas, including frontal regions (i.e. ACC, OFC), ventral striatum, hypothalamus, sensory areas and dopaminergic midbrain (Price, 2003). Via these connections, the amygdala is central to the acquisition, storage and expression of emotional memory (LeDoux, 2000). As the amygdala is implicated in associating external stimuli with aversive or appetitive outcomes, the reaction of the amygdala to conditioned drug cues reflects their emotional valence as a result of pairing with drug use. Inhibition of the amygdala, normally mediated by the PFC, is important in preventing overexpression of these conditioned responses and may be deficient during drug abuse (Quirk and Gehlert, 2003).

6 Expectancy

6.1 Role of Expectancy in Natural and Monetary Reward

The response to environmental stimuli associated with reward is greatly dependent on contextual factors, such as whether the reward is in fact perceived as available. The neural correlates of expectancy to obtain a reward have been given special interest recently, as demonstrated by recent imaging experiments looking at both natural and monetary reward. In a study by O'Doherty et al. (2002), anticipation of a pleasant primary reward (glucose) preferentially activated the OFC, amygdala, VTA, and striatum. In studies involving expectation of monetary reward, similar areas were activated during the state of anticipation, including the ventral striatum (Breiter et al., 2001; Knutson et al., 2001), amygdala, (Breiter et al., 2001) and OFC (Breiter et al., 2001). Taken together, it would seem that expectation of reward, whether natural or conditioned, involves activation of various regions of the mesocorticolimbic DA system. These data seem to support electrophysiological reports on the nature of DA neurons, which show that their firing reflects differences between the predicted outcome and the actual receipt of the reward (Schultz et al., 1998). Involvement of the OFC in the expectation of reward is consistent with its role in mediating the behavioural response to rewarding stimuli in light of the present circumstances (e.g. whether the reward is available or not). The OFC seems to be involved in both the expectancy and receipt of reward (although this may involve distinct sub-regions of the OFC; O'Doherty et al., 2002). This stands to reason as sensory information of both stimuli predictive of the reward, and the reward itself, should be necessary for efficient reward learning in the OFC to occur.

6.2 Role of Expectancy in Drug Abuse

As with stimuli predictive of natural reward, expectancy is an important modulator of the response to drug cues. For example, the impact of expectancy on cue-induced craving has been studied in cigarette smokers (Droungas et al., 1995; Juliano and Brandon, 1998), in which it was shown that craving in response to smoking cues was greater when subjects were anticipating smoking, compared to when a cigarette was not available. In addition to the subjective response to smoking cues, autonomic responses such as salivation and sweating (Carter and Tiffany, 2001; Field and Duka, 2001) are also augmented when smokers are anticipating a cigarette. Although these studies suggest that subjective and physiological measures of cue reactivity are increased with perceived availability, the impact of expectancy on the neural correlates of the response to drug cues has yet to be directly studied. However, a recent analysis of brain imaging studies of cue-induced drug craving suggests that expectancy may be associated primarily with the prefrontal cortex (Wilson et al., 2004). In this examination, it was revealed that those studies exhibiting activation of the DL-PFC and OFC were almost exclusively those involving current drug users, in which the expectancy to use could be assumed. In contrast, those studies of drug users seeking treatment (who were presumably in a state of non-expectancy) did not involve activation of these prefrontal areas, suggesting that the PFC is central to the processing of drug cues in the context of expectancy.

7 Abstinence

Another aspect of the response to drug cues that remains poorly understood from a neural systems approach is abstinence. As with expectancy, abstinence from smoking

is an important factor in nicotine dependence (Solomon and Corbit, 1973). For example, it has been shown that craving during acute withdrawal is strongly linked to the severity of withdrawal symptoms and that alleviation of this craving occurs with smoking (Altman et al., 1996). Generally speaking, nicotine withdrawal symptoms begin after two hours of abstinence, peak between 24 and 48 hours, and can last for up to a month (Miyata and Yanagita, 2001).

To investigate the response to smoking cues during abstinence, a modified Stroop task has been used, in which a smoker must name the colour of a word, meanwhile ignoring the word's semantic content (e.g. "cigarette", "lighter"). A longer delay in the response is considered to be a measure of the salience of the word's meaning (Williams et al., 1996). Studies using this protocol have yielded conflicting results regarding the effect of abstinence on cue reactivity, with evidence of greater response during abstinence (Gross et al., 1993), diminished response (Johnsen et al., 1997; Powell et al., 2002) and no effect (Rusted et al., 2000). However, evidence from both animal work and imaging studies indicates that drug withdrawal may be associated with a disruption of neural systems involved in the response to salient stimuli. In the case of nicotine, it was shown that withdrawal in rats induced an increase in the threshold for intracranial self-stimulation of reward systems (Epping-Jordan et al., 1998), indicative of a hypo-dopaminergic state. Furthermore, imaging studies of various drugs of abuse have showed abnormal brain activity during withdrawal in various mesocorticolimbic regions, including the ACC (van Dyck et al, 1994; Volkow et al., 1993), OFC (Volkow et al., 1991), and striatum (Wu et al., 1997). These findings seem to suggest that the regions

implicated in the detection of, and response to, drug cues are impaired during acute withdrawal.

In the present study, we sought to determine the effect of expectancy and acute withdrawal on the neural response to smoking cues in cigarette smokers. Although the importance of these two features of addiction has been recognized, little is known about their neural correlates. Using fMRI, we measured brain activity while subjects viewed videos alternating between scenes of people smoking and scenes of people getting their hair cut. We varied subjects' degree of expectancy to smoke as they viewed the stimuli, and tested them in two conditions, once after overnight abstinence and once after a period of regular smoking. In addition, we measured the subjective aspect (craving) of cue reactivity during exposure to the cues. The following manuscript details the methodology, results and discussion of this experiment in full.

Expectancy and Abstinence Modulate the Neural Response to Smoking Cues in Cigarette Smokers: an fMRI Study

Dharma T. McBride[1], Andrew Aw[1], Sean P. Barrett[2], Jared T. Kelly[2], Robert Pihl[2], Alain Dagher[1]

[1]*Department of Neurology and Neurosurgery, McConnell Brain Imaging Centre, Montreal Neurological Institute, McGill University, 3801 University, Montreal, QC, Canada, H3A 2B4*

[2] *Department of Psychology, McGill University, 1205 Dr. Penfield, Montreal, QC, Canada, H3A 1B1*

INTRODUCTION

In drug addicts, exposure to drug-associated cues induces both subjective (i.e. craving) and behavioural (i.e. drug-seeking) responses, and is thought to play a significant role in the maintenance of the habit, as well as relapse in those attempting to quit (Abrams et al., 1988). Several functional brain imaging studies have now investigated the neural response to conditioned drug cues in users of various drugs of abuse, including cocaine (Grant et al., 1996; Childress et al., 1999; Garavan et al., 2000), alcohol (Tapert et al., 2003), opiates (Sell et al., 2000; Daglish et al., 2001), and nicotine (Due et al., 2002; Brody et al., 2002). Taken together, these studies reveal that conditioned cues elicit activation of a neural circuitry that may encode the motivational and emotional value that the drug has acquired, and are known to play a role in the planning and control of behavior. The brain regions that have been most commonly implicated in this network include the anterior cingulated (ACC), orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DL-PFC), amygdala, and insula (Wilson et al., 2004). However, many factors that are believed to influence this neural response to drug cues, such as perceived drug availability and the user's level of abstinence, have yet to be investigated.

Examination of the effect of expectancy state on cue-induced craving has shown that smokers report greater urge to smoke when they are anticipating a cigarette, relative to when they are not (Droungas et al., 1995; Juliano and Brandon, 1998). This state of expectancy is believed to be associated with appetitive craving, characterized by the desire for the positive aspects of drug use, such as euphoria and elevated alertness (Tiffany and Drobes, 1991). Although the neural correlates of this form of craving are

unknown, we predicted that cue-associated anticipation and planning of imminent drug use would be mediated by the prefrontal cortex, an executive brain region whose role in addiction is only beginning to be revealed (Wilson et al., 2004; Goldstein and Volkow, 2002). For example, the OFC, a frontal area important in the processing and proper response selection to rewarding stimuli (Rolls, 2000), has been implicated in the expectation of monetary (Breiter et al., 2001), drug (Volkow et al., 2003) and primary (O'Doherty et al., 2002) reward in humans.

Abstinence from chronic drug use is associated with an aversive form of craving, in which the drug is desired so as to alleviate uncomfortable withdrawal symptoms, such as dysphoria and anxiety (Tiffany and Drobes, 1991). It has been well established that nicotine withdrawal increases levels of craving (Shiffman and Jarvik, 1976)), but reports of the effect of abstinence on attentional bias towards smoking cues have yielded conflicting results (Johnsen et al., 2001; Gross et al., 1993). Although imaging studies have suggested that drug withdrawal is associated with regionalized decreases in brain activity (van Dyck et al, 1994; Wu et al., 1997), the neural correlates of cue reactivity during abstinence remain poorly understood.

In the present study, we have used functional magnetic resonance imaging (fMRI) and a cue reactivity paradigm to determine how expectancy and abstinence modulate the neural response to smoking stimuli in cigarette smokers.

METHODS

Participants

Twenty healthy regular smokers (10 men and 10 women; mean age = 27, SD = 8) participated in this study, all smoking at least 15 cigarettes/day (mean = 22, SD = 6 cigarettes/day). All subjects were recruited through McGill University classified ads, or by word of mouth. Subjects gave written consent to the study, in accordance with the Montreal Neurological Institute Research Ethics Board, and were monetarily compensated upon completion of the study. The extent of the smoking habit was quantified using the Fagerstrom Test for Nicotine Dependence (Heatherton et al., 1991), in which a minimum score of 5 (out of a possible 10) was necessary for inclusion into the study. Exclusion criteria included any personal or familial DSM-IV Axis I disorder, neurological illness, previous head trauma, or history of drug abuse (other than nicotine). All subjects, with the exception of two, were right-handed. Data from one female subject was excluded due to abnormal brain MRI; therefore results are based on data from remaining 19 subjects.

Study Design

Each subject was scanned twice, once in an abstinent state (ABS) and once in a non-abstinent state (NON-ABS). The order of scans was randomized and counter-balanced. The ABS scans required subjects to refrain from smoking for 12 hours prior to the scan whereas for the NON-ABS scans, subjects were told to smoke as per their regular habit. To minimize both association effects between scans and effect of variability in circadian or hormonal cycles on cue reactivity, all scans were performed in early afternoon and were separated by approximately 4 weeks (mean = 30, SD = 5 days). Compliance with an abstinent state was verified using a carbon monoxide (CO) monitor

(Vitalograph, Buckingam, UK), which measures the amount of exhaled CO (in parts per million, ppm) as an indicator of the time since the last cigarette. A CO reading of less than 15 ppm indicated that subjects had been compliant with the abstinent state. Subjects were randomly assigned into either the expectant group (EXP) or the non-expectant group (NON-EXP). EXP subjects were told that they would be allowed to smoke a cigarette immediately after the scan, whereas NON-EXP subjects were told that they would have to abstain from smoking for three hours after the scan (compliance verified by CO measurement). Subjects in the EXP and NON-EXP groups did not differ significantly in number of years smoking, cigarettes/day, Fagerstrom test scores, or on any of the personality subscales of the Tri-Dimensional Personality Questionnaire (TPQ; Cloninger et al., 1991). Subjects in the EXP group were on average slightly older than those in the NON-EXP group (EXP: 30, SD = 9; NON-EXP: 24, SD = 5).

On the day of the experiment, prior to the scan, subjects either smoked a cigarette (NON-ABS scan) or were tested for compliance with a 12-hour abstinent state (ABS scan). The Profile of Mood States (POMS; McNair, 1992) was administered to provide a measure of each subject's mood. Subjects were then placed on the MRI scanner bed with their head secured using a vacuum bag to prevent movement throughout the scan. Ear plugs and headphones were administered so as to minimize noise distraction from the scanner.

Stimulus Presentation

Stimulus presentation and response input were coordinated using Media Control Function software (Digivox, Montreal). The behavioural paradigm is summarized in

Figure 1. Visual stimuli in video format were projected onto a screen at the subjects' feet, and were viewable by means of a mirror mounted on the MRI head coil. Six two-minute videos, alternating between smoking (S) and neutral (N) content, were presented in the following order: N-S-S-N-N-S. Neutral stimuli consisted of people getting their hair cut, whereas smoking stimuli involved people engaged in smoking behaviour (i.e. lighting cigarettes, smoking while socializing, blowing smoke rings, etc.). These video stimuli had been validated in a previous study showing that the S videos reliably induced craving for cigarettes in a separate set of smokers (data not shown). Videos were controlled for degree of facial exposure, movement and physical characteristics of the actors. Subjects responded to craving questionnaires projected on the screen using a computer mouse to slide a cursor to the appropriate point on a visual analog scale (ranging from 0 to 10). The seven-item questionnaire was given seven times: once before the stimuli (baseline) and once after each of the six videos. A one minute baseline target screen (white cross on a grey background) preceded the onset of the videos and gave a pre-stimulus measure of resting brain activity. In addition, each video was preceded by a 20 second target screen, which provided a distraction to minimize craving run-over between sessions as well as a cue-independent state to quantify any such run-over.

Self-reports of craving

Each video was followed by seven questions pertaining to various aspects related to craving. All questions were subjectively answered on a scale, ranging from 0 ("not at all") to 10 ("extremely"). The questions included: one general craving question ("I am craving a cigarette right now"), two questions regarding positive aspects of craving ("A

cigarette would taste good right now" and "A cigarette would feel good right now"), two questions regarding the negative aspects of craving ("I am feeling irritable right now" and "I could think clearer if I could smoke a cigarette right now"), one question concerning the intention to smoke ("If it were possible, I would smoke a cigarette right now"), and one question gauging the subject's boredom during the previous video ("I felt bored during the last video clip"). Questions 1 to 6 were based on items of the Questionnaire of Smoking Urges (Tiffany and Drobes, 1991).

Imaging Parameters

All imaging scans were carried out at the Montreal Neurological Institute using a 1.5 Tesla Siemens Sonata MRI scanner. Each session began with the acquisition of a series of 160 high-resolution (1mm), T1-weighted sagittal images for anatomical localization of the functional data. Blood oxygen level dependent (BOLD) functional MRI was employed to obtain task-dependent neural activity, using a gradient-echo, echo-planar imaging (EPI) sequence (TR, 4000 ms; pause after measurement, 400 ms; TE, 50 ms; flip angle, 90^{o}). Each session included one functional scan (approximately 21 minutes long), in which 302 continuous acquisitions were taken. Each acquisition consisted of thirty-six T2*-weighted, contiguous slices (voxel size, 4 mm X 4 mm X 4 mm) parallel to the anterior commissure-posterior commissure axis so as to allow for full brain coverage.

fMRI Data Analysis

Functional images were blurred with a 6 mm full-width at half-maximum Gaussian filter and were corrected for motion and head drift using the fourth frame as reference (the first three were discarded as the BOLD signal had yet to reach steady state). Frames showing excessive movement were identified visually and excluded from the data set before statistical analysis. Functional BOLD data was analyzed using fmristat software (for full description of statistical method, see Monchi et al., 2004; Worsley et al., 2002; available at http://www.bic.mni.mcgill.ca/users/keith). Signal drift of first order linear extent was removed from the functional data. In order to combine multiple data sets, both functional EPI images and anatomical images were mapped into the standard stereotaxic space of Talairach and Tournoux using a customized algorithm (Collins et al., 1994). For individual sessions, all 30 frames of BOLD data associated with each separate 2 minute video were averaged, then voxel-wise comparisons between adjacent neutral and smoking stimuli (runs) were performed (i.e. S_1-N_1, S_2-N_2, S_3-N_3), and then the three runs were averaged (S_{AVG}-N_{AVG}). Using the resulting effect and standard deviation effect images, statistical T maps were generated for multiple scans using a mixed effects linear model for the effects (as data) with fixed effects standard deviations taken from the previous analysis. To determine the overall effect of smoking stimuli on neural activity in cigarette smokers, data from all sessions and subjects were combined. The effects of expectancy (between-subject factor; EXP vs. NON-EXP) and abstinence (within-subject factor; ABS vs. NON-ABS) on this response were investigated by combining data across groups and across sessions, respectively. To determine how these states significantly differed from each other, subtractions between each state were also performed (i.e. EXP – NON-EXP; ABS – NON-ABS). T maps were thresholded for

statistical significance using the Bonferroni method/random field theory correction for multiple comparisons. A single voxel was deemed significant if its t-value exceeded 4.95, based on a grey matter volume of 600 mm^3. Significance was also assessed on the basis of the spatial extent of consecutive voxels, such that a cluster with volume >352 mm^3 and $t > 3$ was significant ($p<0.05$) when corrected for multiple comparisons. In addition, certain regions were predicted *a priori* to show activation in response to smoking cues based on previous literature and were considered significant at a threshold of $p<0.001$ uncorrected. These predicted regions included frontal areas (DL-PFC and medial prefrontal cortex (mPFC), ACC, OFC), limbic system (dorsomedial thalamus, amygdala, hippocampus, parahippocampal gyrus, temporal pole, ventral striatum, ventral tegmental area), and visuospatial areas (primary and association visual cortices, posterior parietal cortex). For all areas showing significant activation in the smoking – neutral contrasts, as well as *a priori* regions, correlations between relative change in BOLD signal and craving scores were performed using the least squares linear regression method. A voxel-based correlation analysis was performed to determine functional connectivity for regions of interest. Peak voxels (from smoking – neutral t maps) were extracted and a brain-wide search was performed for areas whose BOLD signal covaried significantly with BOLD in that voxel. This was done for each expectancy group and abstinent condition separately, as well as for all subjects combined.

Time courses of the BOLD signal for three regions (ACC, DL-PFC, medial temporal lobe/ventral pallidum (MTL/VP)) were performed by extracting the voxels shown to respond to smoking cues in the EXP group (for the ACC and DL-PFC) or NON-ABS session (for the MTL/VP). All subjects' functional data was transformed into

Talairach space, and then combined to provide a temporal measure of the BOLD signal throughout the stimulus presentation sequence. In addition, BOLD signal values were extracted for these regions and relative change between neutral and smoking values was determined.

RESULTS

Subjective self-reports

Abstinence from smoking significantly decreased self-reported baseline measures of mood and alertness on the POMS. Relative to their non-abstinent session, abstinent subjects reported increased anxiety (composed/anxious: ABS: mean=19.9, SE=1.8; NONABS: mean=26.4, SE=0.9; p=0.001), depression (elated/depressed: ABS: mean=21.9, SE=1.3; NONABS: mean=27.8, SE=1.0; p<0.001), fatigue (energetic/tired: ABS: mean=19.1, SE=2.0; NONABS: mean=24.1, SE=1.3; p=0.013), hostility (agreeable/hostile: ABS: mean=23.5, SE=1.6; NONABS: mean=30.3, SE=1.0; p<0.001), insecurity (confident/unsure: ABS: mean=23.0, SE=1.3; NONABS: mean=26.1, SE=0.7, p=0.007), and confusion (POMS clear/confused: ABS: mean=22.9, SE=1.9; NONABS: mean=28.9, SE=0.8; p=0.001). In addition, subjects craved cigarettes more at baseline when abstinent than when non-abstinent (ABS: mean=51.1, SE=3.4; NONABS: mean=40.2, SE=3.1; p=0.02). However, there was no significant difference in baseline craving between expectancy groups when abstinence sessions were combined (EXP: mean=43.4, SE=3.2; NONEXP: mean=47.9, SE=3.8; p=0.36). A mixed ANOVA (cue X abstinence X expectancy) was performed on the craving self-reports following each video clip for each questionnaire item separately (see Table 1 for data and statistics). When

compared to the neutral videos, smoking videos were successful in significantly increasing subjective reports of general craving, a finding that was evident for each group-session separately (Figure 2B). Similar results were seen for positive and negative indices of craving, and intention to smoke, with the exception of boredom, in which the opposite effect was found (i.e. boredom greater during neutral videos). Relative to the non-abstinent state, subjects gave significantly higher reports of general craving when abstinent from cigarettes. A similar trend existed for other indices, with the exception of boredom, which did not differ between states. There was also a significant main effect of expectancy on reports of general craving and boredom, with non-expectant subjects reporting higher levels of these two indices than those subjects expecting to smoke after their scan. A significant interaction between abstinence and cue revealed that subjects' greater degree of irritability after smoking cues than neutral cues was higher during abstinence than non-abstinence from smoking.

BOLD response to smoking stimuli

All Subjects:

Table 2 lists brain regions showing BOLD signal change between neutral (N) and smoking (S) stimuli for all subjects and conditions combined. Relative to neutral stimuli, smoking stimuli induced activations in left ACC, mPFC, left medial OFC (mOFC), left posterior cingulate cortex (PCC), left anterior insula, right middle/superior temporal gyrus, bilateral precuneus, and left dorsomedial thalamus. The reverse subtraction disclosed a wide array of regions activated by the neutral videos in comparison to the smoking videos. These areas include the premotor cortex, supplementary motor area,

posterior insula, and a large cluster composed of areas of the postcentral sulcus, intraparietal sulcus, superior parietal lobule, superior and middle occipital gyri, fusiform gyrus and inferior temporal gyrus. These activations were all bilateral and robust, and were also present for both expectancy groups, both abstinence sessions, and were evident for every scanning run (i.e. S_1-N_1, S_2-N_2, S_3-N_3).

Expectant vs. Non-Expectant:

Regions activated by smoking stimuli differed between expectancy groups, as demonstrated in Table 3 and Figure 2A. In response to smoking stimuli (i.e. smoking – neutral), subjects in the expectant state (both sessions combined) showed activation of frontal regions (bilateral ACC, bilateral mPFC, left PCC, left mOFC, and bilateral DL-PFC), left anterior insula, right posterior parietal cortex, bilateral cuneus, left precuneus, and left dorsomedial thalamus. In sharp contrast, subjects in the non-expectant group showed activation solely in the right middle/superior temporal gyrus in response to smoking stimuli. When smoking cue-associated activation was contrasted between groups, the expectant group exhibited significantly higher activation of the left DL-PFC (i.e. EXP > NONEXP; Figure 3A), whereas there was greater activation of the right mOFC in the non-expectant group (ie. NONEXP > EXP). Figure 5A shows the time course of the BOLD signal for the left ACC and DL-PFC regions for both the EXP and NON-EXP groups, demonstrating the difference in the preferential response to smoking cues during expectancy. Figure 5B illustrates this graphically, in which a greater relative change in this BOLD signal (smoking vs. neutral) during an EXP condition is evident for the ACC and DL-PFC.

A significant positive correlation between general craving and BOLD response in the left DL-PFC was found for the EXP group ($p=0.04$), whereas a trend towards a negative correlation was found for the NON-EXP group ($p=0.12$; Figure 3B). Smoking cue-induced (i.e. smoking – neutral) BOLD signal in the left ACC (peak voxel: x=-4 y=30, z=16) covaried positively with BOLD signal in the right midline thalamus (x=8, y=-12, z=4) for the EXP group ($t=4.31$, $p<0.05$), but not for the NON-EXP group ($t=2.18$, NS).

Abstinent vs. Non-Abstinent:

As with expectancy state, there was a large effect of abstinence on subjects' response to smoking stimuli (Table 4 and Figure 2A). Relative to neutral stimuli, abstinent smokers exposed to smoking cues (both groups combined) showed activation of the left ACC, though this was only statistically significant when uncorrected for multiple comparisons (i.e. as an *a priori* region). Smokers in a non-abstinent state also had activation of the left ACC, but, in addition, exhibited activation of the right PCC, left precuneus, right middle temporal gyrus, left dorsomedial thalamus and a region including parts of the right medial temporal lobe and ventral pallidum (MTL/VP, Figure 4). When abstinence states were contrasted, smoking cue-induced activation of the right MTL/VP was shown to be significantly greater during the non-abstinent state than during the abstinent state (Table 4 and Figure 5B).

Table 1. Cigarette smokers' subjective self-reports of various indices of craving for neutral and smoking stimuli, during each expectancy state and abstinence condition.

QUESTIONNAIRE ITEM	EXPECTANT (n=10)				NON-EXPECTANT (n=9)				ALL SUBJECTS (n=19X2)	
	ABS		NONABS		ABS		NONABS			
	N	S	N	S	N	S	N	S	N	S
I am craving a cigarette right now € ★ ✦	5.7 (±0.6)	6.9 (±0.6)	4.2 (±0.6)	5.1 (±0.5)	6.1 (±0.5)	7.5 (±0.5)	4.9 (±0.7)	6.4 (±0.7)	5.2 (±0.3)	6.5 (±0.3)
A cigarette would feel good right now € ★	6.1 (±0.6)	7.0 (±0.6)	4.4 (±0.7)	5.4 (±0.7)	6.5 (±0.5)	7.6 (±0.5)	5.4 (±0.7)	6.6 (±0.7)	5.7 (±0.3)	6.6 (±0.3)
A cigarette would taste good right now € ★	6.2 (±0.4)	7.2 (±0.4)	4.5 (±0.6)	5.7 (±0.7)	6.4 (±0.5)	7.6 (±0.4)	5.4 (±0.7)	6.6 (±0.7)	5.6 (±0.3)	6.8 (±0.3)
I am feeling irritable right now € ★ †	5.1 (±0.8)	5.5 (±0.8)	3.5 (±0.6)	3.8 (±0.6)	5.2 (±0.7)	5.9 (±0.8)	4.0 (±0.8)	3.9 (±0.8)	4.4 (±0.4)	4.8 (±0.4)
I could think more clearly if I had a cigarette right now € ★	6.0 (±0.7)	6.5 (±0.7)	4.1 (±0.6)	4.6 (±0.6)	5.6 (±0.7)	6.1 (±0.8)	4.8 (±0.8)	5.1 (±0.8)	5.1 (±0.4)	5.6 (±0.4)
If it were possible, I would smoke a cigarette right now € ★	7.2 (±0.5)	8.2 (±0.5)	5.0 (±0.7)	6.5 (±0.7)	7.2 (±0.5)	8.0 (±0.5)	6.1 (±0.8)	7.0 (±0.7)	6.4 (±0.3)	7.4 (±0.3)
I felt bored during the last video clip € ✦	4.6 (±0.7)	3.3 (±0.5)	5.1 (±0.8)	3.5 (±0.7)	6.1 (±0.7)	4.2 (±0.6)	5.6 (±0.8)	3.8 (±0.9)	5.3 (±0.4)	3.7 (±0.3)

N=Self-report measures associated with neutral stimuli; S= Self-report measures associated with smoking stimuli; All reports scored on a scale ranging from 0 ("not at all") to 10 ("extremely"); Numbers in brackets denote standard error of the mean (SEM); €: Significant main effect of cue on self-report ("craving": $F=94.5$, $p<0.001$; "feel good": $F=56.2$, $p<0.001$; "taste good": $F=98.9$, $p<0.001$; "irritable": $F=5.1$, $p=0.027$; "think clearer": $F=14.1$, $p<0.001$; "would smoke": $F=78.7$, $p<0.001$; "bored": $F=35.5$, $p<0.001$;df=1,58); ★: Significant main effect of abstinence on self-report ("craving": $F=34.0$, $p<0.001$; "feel good": $F=37.1$, $p<0.001$; "taste good": $F=35.0$, $p<0.001$; "irritable": $F=28.3$, $p<0.001$; "think clearer": $F=27.2$, $p<0.001$; "would smoke": $F=39.4$, $p<0.001$; "bored": $F=0.02$, $p=0.889$ (NS); df=1,58); ✦: Significant main effect of expectancy on self-report ("craving": $F=3.8$, $p=0.055$; "bored": $F=3.9$, $p=0.054$; df=1,58); † : Significant interaction between abstinence and cue ("irritable": $F(1,58)=4.8$, $p=0.033$).

TABLE 2. Brain regions of significant BOLD signal contrasts between neutral and smoking videos for all subjects combined.

Region		Cluster Size (mm^3)	Peak Voxel				Cluster p-Value
			x	y	z	t-Value	
Smoking > Neutral							
Anterior cingulate cortex (24/32)	L	9184	-4	30	24	5.35	<0.001
	L		-4	40	8	5.21	
Medial prefrontal cortex (9/10)	L/R		0	56	22	4.52	
Posterior cingulate cortex (23)	L	1712	-2	-16	36	4.27	<0.001
Medial orbitofrontal cortex (11)	L	288	-8	60	-14	4.07	0.032*
Superior temporal gyrus (21/22)	R	2448	50	-34	2	4.93	<0.001
Anterior insula (13)	L	1000	-36	12	2	4.30	0.011
Precuneus (7)	L	968	-8	-58	36	4.60	0.013
	R		4	-58	36	3.64	
Dorsomedial thalamus	L	336	-2	-6	6	4.56	0.022*
Neutral > Smoking ✦							
Precentral gyrus (6)	L/R	4136	-56	2	34	6.65	<0.001
	L/R	5616	-26	-14	54	7.76	<0.001
Posterior insula (13)	L/R	1376	-38	-6	16	5.94	0.002
Intraparietal sulcus (40/7)	L/R	66092	-40	-45	58	11.08	<0.001
Postcentral sulcus (40/2)	L/R		-58	-26	35	9.32	
Superior parietal lobule (7)	L/R		-26	-61	59	8.07	
Superior occipital gyrus (19)	L/R		-22	-81	38	6.97	
Middle occipital gyrus (19)	L/R		-35	-81	10	5.99	
Fusiform gyrus (37)	L/R		-26	-52	-13	6.11	
L/R Inferior temporal gyrus (37)	L/R		-48	-68	-2	8.65	

Numbers in brackets refer to Brodmann's areas; L, Left; R, Right; ✦: All regions were bilateral, however, only coordinates and statistics shown for left side; * : p-Value uncorrected (*a priori* region of interest)

TABLE 3. Brain regions of significant activation in response to smoking stimuli (> neutral stimuli) for expectant (n=10) and non-expectant (n=9) groups, as well as for between groups.

Region		Cluster Size (mm^3)	Peak Voxel				Cluster p-Value
			x	y	z	t-Value	
EXP (S>N)							
Anterior cingulate cortex (24/32)	L	2688	-16	-8	26	4.82	<0.001
	L	15976	-4	30	16	5.82	<0.001
	L		-10	24	30	5.61	
	R		6	46	8	4.25	
Medial prefrontal cortex (9/10)	L/R		0	58	20	4.88	
	R		2	32	32	4.19	
Posterior cingulate cortex (23)	L		-4	-24	26	4.12	
Medial orbitofrontal cortex (11)	L	464	-8	60	-14	4.11	0.009*
Dorsolateral prefrontal cortex (8/9)	L†	248	-30	36	42	4.19	0.045*
	R	288	20	58	34	4.12	0.032*
Posterior parietal cortex (39/40/7)	R	1784	58	-56	24	4.41	<0.001
Cuneus (17/18/19)	R	1736	6	-74	32	4.07	0.001
	L		-4	-74	26	3.65	
Precuneus (31)	R		8	-58	22	3.24	
Anterior insula (13)	L	416	-38	16	4	3.92	0.012*
Dorsomedial thalamus	L	312	-4	-6	6	4.40	0.027*
NONEXP (S>N)							
Superior temporal gyrus (21/22)	R	1888	54	-36	4	4.57	<0.001
EXP > NONEXP (S>N)							
Dorsolateral prefrontal cortex (8/9)	L	504	-40	34	38	4.52	0.007*
NONEXP > EXP (S>N)							
Medial orbitofrontal cortex (11)	R	248	12	50	-20	3.82	0.044*

S: BOLD signal associated with smoking stimuli; N: BOLD signal associated with neutral stimuli; Numbers in brackets refer to Brodmann's areas; L, Left; R, Right; *: p Value uncorrected (*a priori* region of interest); † : positive correlation between general craving and BOLD response (p=0.04).

TABLE 4. Brain regions of significant activation in response to smoking stimuli (> neutral stimuli) for abstinent (n=18) and non-abstinent (n=18) sessions, as well as for between sessions.

Region		Cluster Size (mm^3)	Peak Voxel				Cluster p-Value
			x	y	z	t-Value	
ABS (S>N)							
Anterior cingulate cortex (32)	L	424	-10	16	32	3.62	0.012*
NON-ABS (S>N)							
Anterior cingulate cortex (24/32)	L	4696	-2	28	18	4.57	<0.001
Anterior cingulate cortex (24)	L	640	-2	-16	38	3.66	0.003*
Posterior cingulate cortex (23)	R	928	10	-20	26	4.00	0.015
MTL/ventral pallidum	R	1720	18	-10	-8	5.17	<0.001
Dorsomedial thalamus	L	248	-2	-8	6	3.75	0.045*
Precuneus (31/7)	L	864	-8	-58	36	3.94	0.022
Middle temporal gyrus 21)	R	728	50	-32	2	4.14	0.046
NON-ABS > ABS (S>N)							
MTL/ventral pallidum	R	256	12	-2	-10	4.12	0.042*

S: BOLD signal associated with smoking stimuli; N: BOLD signal associated with neutral stimuli; Numbers in brackets refer to Brodmann's areas; L, Left; R, Right; *: p Value uncorrected (*a priori* region of interest); † : positive correlation between general craving and BOLD response (p=0.04); MTL, medial temporal lobe.

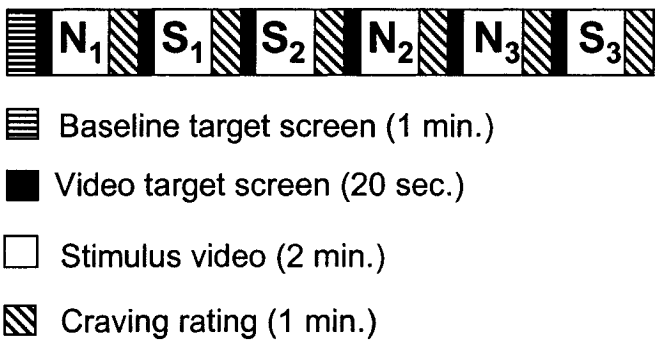

FIGURE 1. Stimulus presentation design showing alternation between smoking (S) and neutral (N) videos, with craving questionnaire following each video.

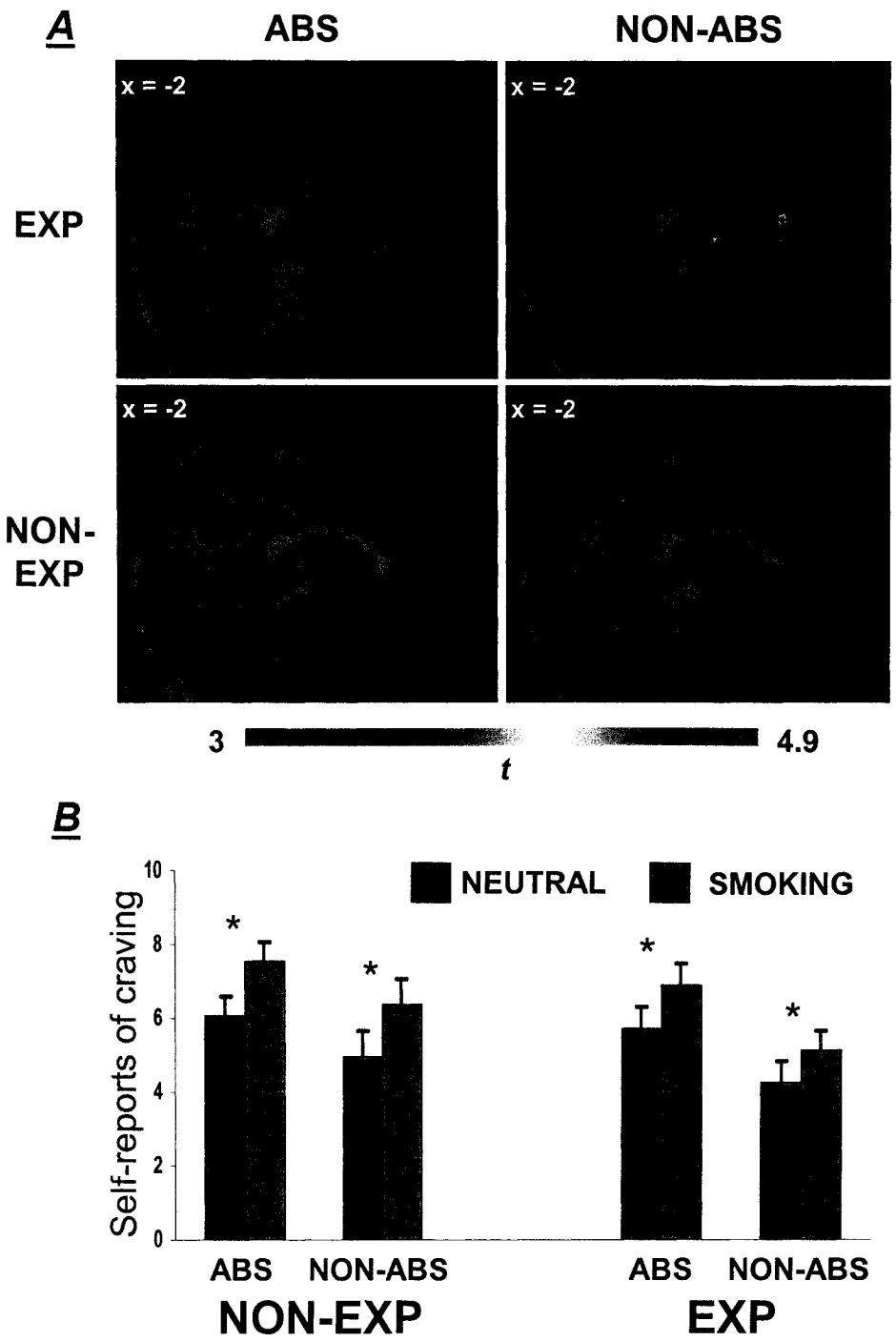

FIGURE 2. *A*, T maps showing neural response to smoking cues (relative to neutral cues) in each of the expectancy and abstinence conditions. Note the preferential response to smoking cues during an expectant and non-abstinent state. ***B***, Subjective self-reports of craving after neutral and smoking videos, in each of the expectancy states and abstinence conditions. Note the greater craving response to smoking cues and during an abstinent state. ABS, abstinent; NON-ABS, non-abstinent; EXP, expectant; NON-EXP, non-expectant; * : $p < 0.01$. Error bars represent standard error (SE) of the mean.

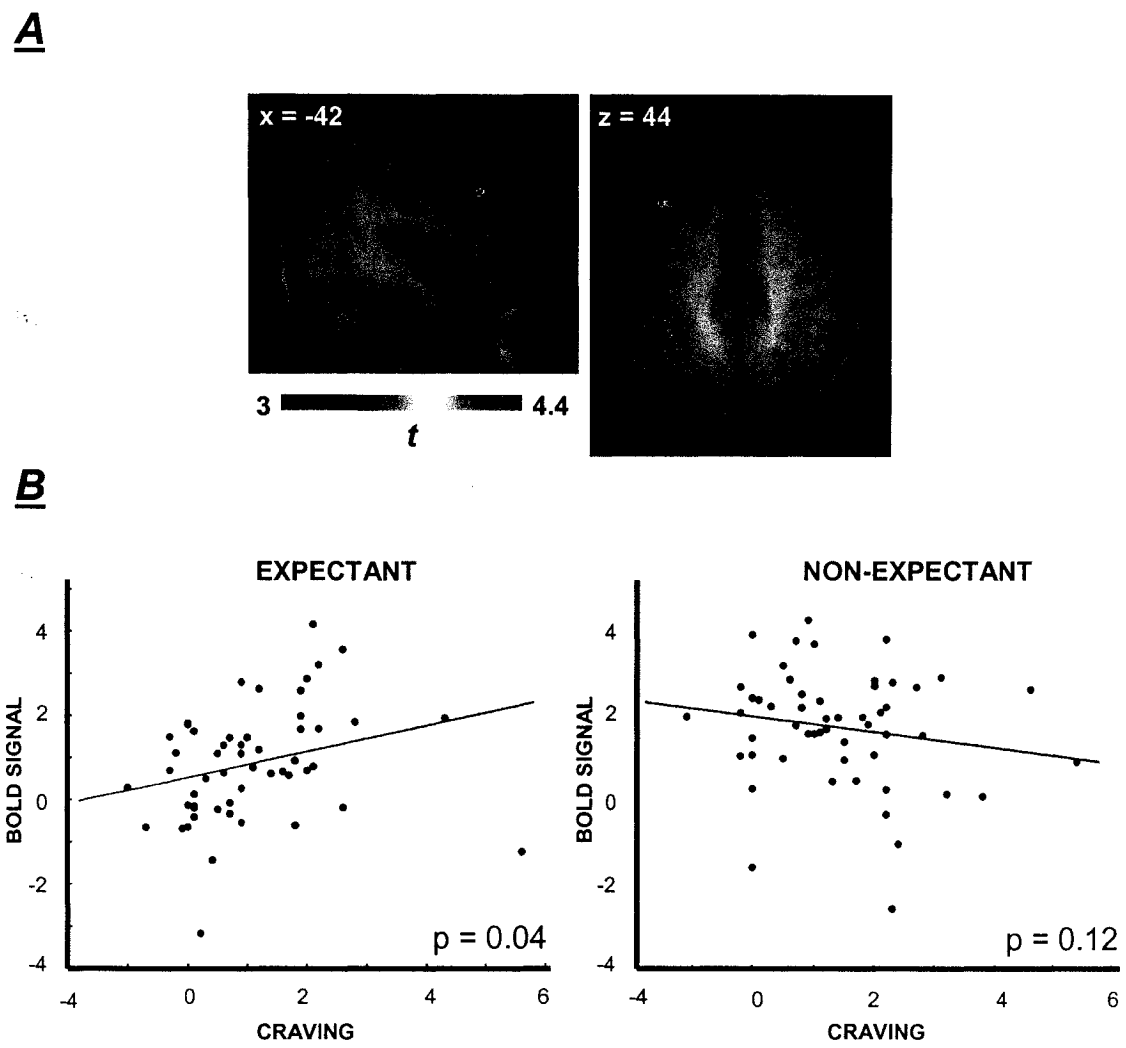

FIGURE 3. *A*, T maps showing activation of the left dorsolateral prefrontal cortex (DL-PFC) in response to smoking cues (relative to neutral cues) during an expectant state. ***B***, Correlations between BOLD signal in the left DL-PFC and self-reports of general craving (smoking – neutral) during the expectant and non-expectant states. Note the positive correlation during an expectant state (p=0.04) and the trend towards a negative correlation during a non-expectant state (p=0.12).

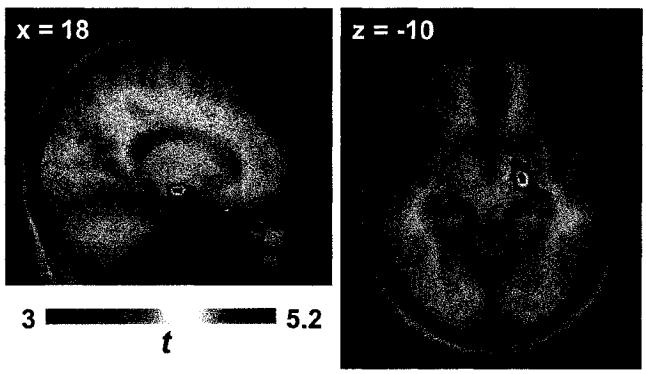

FIGURE 4. T maps showing activation of the right medial temporal lobe/ ventral pallidum (MTL/VP) in response to smoking cues (relative to neutral cues) during a non-abstinent state.

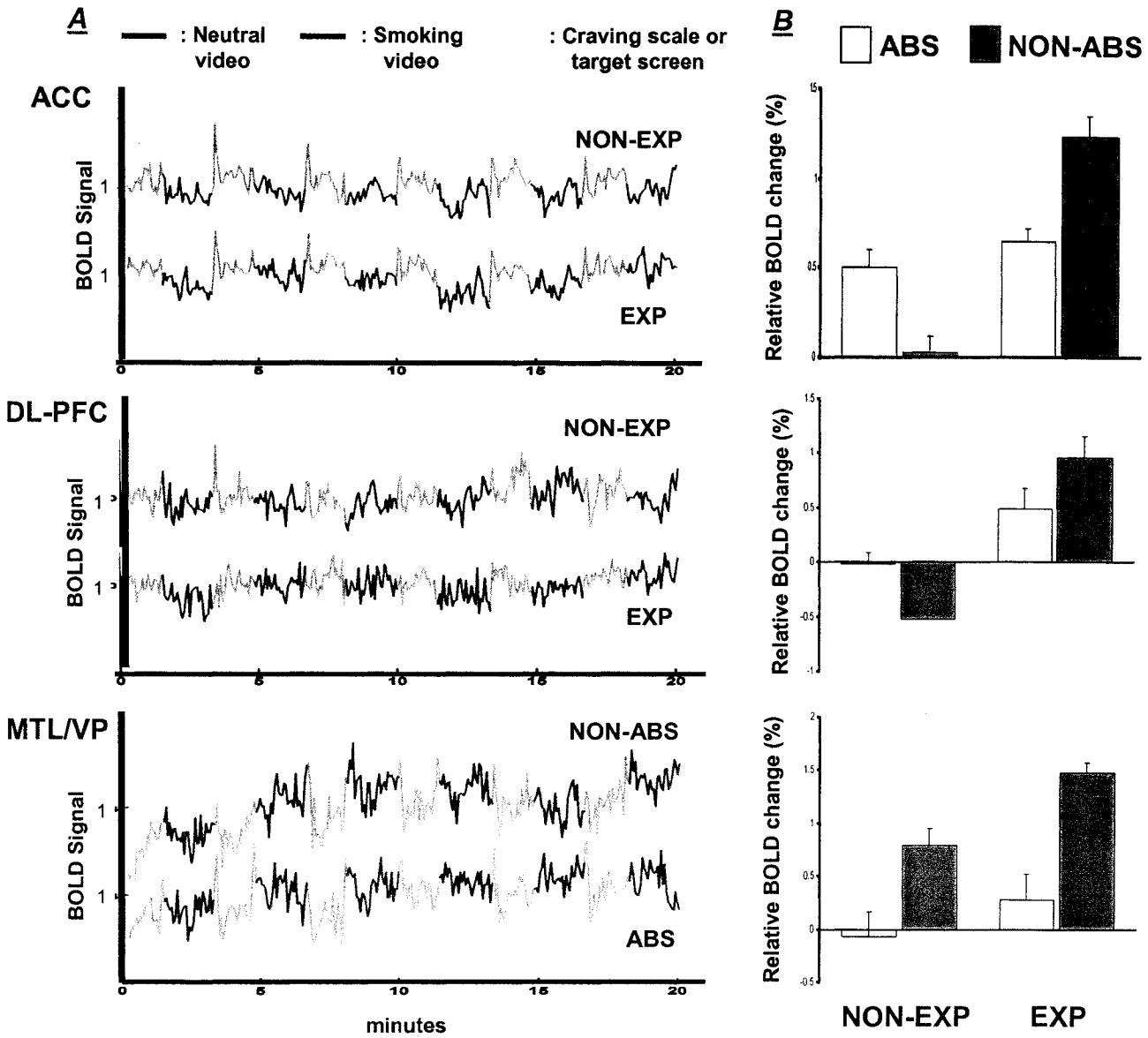

FIGURE 5. *A*, Time courses of BOLD signal for anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DL-PFC) and medial temporal lobe/ ventral pallidum (MTL/VP) contrasting expectancy states or abstinence conditions. Note the increase in signal during the smoking videos for the EXP but not the NON-EXP group in the ACC and DL-PFC, and for the NON-ABS but not the ABS condition in the MTL/VP. ***B***, Graphs of relative BOLD signal change between smoking and neutral stimuli for ACC, DL-PFC and MTL/VP for each of the expectancy states and abstinence conditions. ABS, abstinent; NON-ABS, non-abstinent; EXP, expectant; NON-EXP, non-expectant. Error bars represent standard error (SE) of the mean.

DISCUSSION

In the present study, we have demonstrated that cigarette smokers exposed to smoking stimuli activate neural circuitry that is consistent with previous studies of cue-induced drug craving and likely reflects the learned motivational salience of the stimuli. Cigarette smokers responded to smoking stimuli with increased reports of various indices of craving, as well as activation of a distributed neuronal network, including the anterior (ACC) and posterior cingulate cortex (PCC), medial prefrontal cortex (mPFC), medial orbitofrontal cortex (mOFC), anterior insula, middle/superior temporal gyrus, precuneus, and dorsomedial thalamus. However, we report here, for the first time, that the neurophysiological response to conditioned cues is strongly modulated by both perceived drug availability and level of abstinence.

Based on the results shown here, we claim that expectancy is a crucial factor in determining the neural response to conditioned drug cues. Whereas a state of expectancy was associated with cue-induced activation of numerous cortical regions (ACC, mPFC, mOFC, dorsolateral prefrontal cortex (DL-PFC), PCC, anterior insula, posterior parietal cortex, cuneus/ precuneus), and the dorsomedial thalamus (relay nucleus for the PFC), only the middle/superior temporal gyrus was specifically activated by smoking cues in a non-expectant state. A notable finding of these data is the association of the prefrontal cortex (PFC) only with an expectant state. In light of its mainly executive functions, the expectancy-dependent activation of the PFC in response to smoking cues could represent the processing of cognitive and motivational aspects of stimuli only when they are perceived as relevant (i.e. available).

Although many PFC regions were selectively activated during an expectant state, only the DL-PFC showed cue-induced activation that was significantly greater during expectancy than non-expectancy. Although the executive role of the DL-PFC in planning and working memory has been well documented (Petrides, 2000), implication of a dysfunctional DL-PFC in pathological states such as drug addiction has only recently been addressed (Goldstein and Volkow, 2002; Wilson et al., 2004). A recent analysis of multiple cue-induced drug craving studies revealed that activation of the DL-PFC in response to drug cues was observed only in those studies using subjects not seeking treatment, in which their expectation to obtain and use the drug after the study could be assumed (Wilson et al., 2004). A possible explanation of this expectancy-dependent DL-PFC response to drug cues is that during the course of addiction, the stimuli that reliably predict drug use also become associated with the user's planning and expectation to obtain the drug, a process likely involving the DL-PFC. Thus, by means of a Pavlovian conditioning mechanism, a state of expectancy may itself act as a conditioned stimulus and work in concert with other drug cues to promote drug seeking. An alternative, though not necessarily mutually exclusive, explanation is that knowledge of drug availability could act as a gate for the processing of, and subsequent acting on, conditioned drug cues. In this model, the DL-PFC would mediate top-down processing of external stimuli (i.e. smoking cues) only when the reward they are associated with (i.e. nicotine) was seen as obtainable.

If indeed the DL-PFC is a key neural substrate of expectancy, then it may serve as a useful therapeutic target for addiction. Supporting this notion was recent evidence showing that modulation of DL-PFC activity using repetitive transcranial magnetic

stimulation (rTMS) was sufficient to decrease the rate of smoking in cigarette smokers (Eichhammer et al., 2003). Interestingly, it was found that this intervention did not affect craving for cigarettes, suggesting that the DL-PFC may be more involved in the compulsive aspect of addiction, rather than in conscious craving. In the present study, we found a positive correlation between craving and BOLD response to smoking cues in the left DL-PFC, an effect that was only apparent during an expectant state. Although this could imply that the DL-PFC is implicated in craving, our results suggest that, rather than being an actual locus for craving, this executive region likely functions to incorporate multiple internal (i.e. craving, perceived availability, motivation) and external (i.e. conditioned cues) factors into an appropriate response (i.e. drug-seeking or avoidance). Thus, treatment methods that modulate DL-PFC activity may not influence craving *per se*, but instead may be effective in dampening the behavioural response to drug cues, independently of craving. This would suggest that drug craving represents a motivational emotional state which is but one element influencing goal-directed behaviour, albeit pathologically. It should be noted that the activation of the DL-PFC seen here may also represent a resistance of drug-seeking behaviour, in which it must inhibit normal reflexive response to drug cues. The DL-PFC has been previously implicated in the inhibition of normal response tendencies to visual stimuli (Brass et al., 2001). Interestingly, this DL-PFC activity coexisted with activation in the precuneus and posterior cortex, two areas that were also activated by smoking cues in the present study.

The ACC has been the most commonly reported brain region in imaging studies of cue-induced drug craving (11 of 19; Wilson et al., 2004), and in the present study showed the most widespread activation to smoking stimuli. In light of these previous

findings, evidence shown here of ACC activation following presentation of smoking stimuli suggests that this area may be an important neural correlate of the response to salient stimuli. This is in keeping with its functional connectivity, which includes reciprocal projections with the PFC, limbic areas, motor regions and the dopaminergic midbrain (Paus, 2001). In the present study, the ACC response to cues was very strong during an expectant condition but only marginally significant during a state of non-expectancy to smoke. Due to its extensive connections with other prefrontal areas such as the OFC and DL-PFC, the ACC may have the ability to process salient stimuli in light of their perceived availability. However, an examination of imaging studies of drug cue reactivity reveals that the presence of cue-induced ACC activation cannot be fully accounted for by methodological factors, such as the type of drug dependence, imaging modality or, notably, subjects' treatment status (i.e. perceived drug availability). Therefore, it is unlikely that the ACC activity seen here can be completely attributed to a state of expectation *per se*; rather it may reflect a more general role of this brain area in the regulation of attention and response selection to attended stimuli (Isomura et al., 2003). As such, the greater ACC activity in the EXP state could represent the allocation of more attention to the smoking cues during a state of anticipation to smoke, in which the cues would hold more significance relative to a non-expectant state. Unlike other studies, the association between expectancy and cue-induced ACC activity seen here may have been exceptionally apparent because of the explicit instructions regarding cigarette availability given to the subjects. As such, the lower ACC activation to the smoking videos during a non-expectant state could reflect a conscious inhibition of a normally automatic response to salient cues.

An alternative explanation, in keeping with the ACC's proposed role in conflict monitoring and inhibition of response tendencies (Kerns et al., 2004; Carter et al., 1999), is that the expectancy-dependent ACC activation seen here represents the expectant subjects' conflict at not being able to immediately respond to the conditioned cues, in spite of having the perception of drug availability. Non-expectant smokers would not have experienced this conflict, as smoking was not an option, reflected by a relative absence of cue-induced ACC activation during this state.

Other studies have suggested that the ACC plays a primary role in the regulation of arousal (Paus, 2000). Imaging experiments with humans have shown that activity in the ACC is modulated by the arousal state of the organism, from sleep to wakefulness, and this arousal-related activity covaries with that of the midline thalamus, part of the ascending reticular activating system (Paus, 2000). Interestingly, the present fMRI study provided evidence that such a process may contribute to the preferential attention given to drug cues in addiction. These results showed that smoking cue-induced ACC activity correlated positively with activity in a similar region of the midline thalamus implicated in other studies (Paus, 2000). Thus it would suggest that the ACC activity seen here may be a neural correlate of drug cue-induced increase in arousal. Evidence of the ACC's ability to control autonomic functions such as heart rate, independently of cognitive task (Critchley et al., 2003), corroborate its key role in regulating arousal state. Interestingly, we showed that this regulation is influenced by contextual experience, in that we found significant correlations between smoking cue-induced ACC and midline thalamic activity only during a state of expectancy. This could reflect an anticipation-dependent regulation of arousal that promotes the preparation of action only when it is relevant, such as during

the response to salient cues that signal an available reward. Consistent with this neuronal evidence of greater cue-induced arousal during an expectant state, subjects in this study reported lower degrees of boredom (i.e. increased arousal) during exposure to smoking cues (relative to neutral cues), and during a state of anticipation to smoke. As there is also evidence of an augmentation in cue-induced autonomic response with expectancy (Carter and Tiffany, 2001; Field and Duka, 2001), it would seem that perceived availability can increase levels of arousal in response to drug cues, a process possibly mediated by the ACC.

In the present study, a state of abstinence from smoking was associated with a remarkably blunted neural response to smoking cues throughout the brain, including the ACC. This effect was evident even during a state of expectancy, where activity in this region (and others) was greatest. This result may serve as evidence of a hypodopaminergic state during withdrawal, as the neural activity of the ACC, which receives extensive dopaminergic innervation (Paus, 2000), is believed to be greatly dopamine-dependent (Volkow et al., 2000). In addition to the present study, withdrawal-induced decreases in ACC activity have also been shown in both methamphetamine (London et al., 2004) and opiate (van Dyck et al., 1994) withdrawn patients, suggesting that this effect is common among many drugs of abuse. Indeed, a dysfunctional ACC during withdrawal may disrupt normal attention and response selection processes and could explain the propensity towards relapse in abstinent drug users.

The relative lack of neural activation specific to smoking stimuli during an abstinent state seen here supports previous findings of a diminished response to salient stimuli during acute withdrawal from nicotine (Powell et al., 2002). It has been proposed

that drug withdrawal results in an underactive mesolimbic dopamine system (Spanagel and Weiss, 1999), an idea that is supported in rodents, where spontaneous nicotine withdrawal was shown to decrease brain reward function (Epping-Jordan et al., 1998). A disrupted dopaminergic state during withdrawal could explain our finding that the ventral pallidal region was activated by smoking stimuli only in the non-abstinent state, and that this BOLD response was significantly greater during non-abstinence than during an abstinent state. The ventral pallidum (VP), which receives dopaminergic input from the midbrain (Klitenick et al., 1992), has been shown to be important in the conditioned response to psychostimulants (Gong et al., 1997; Fletcher et al., 1998), though its role in the response to conditioned cues has not been well established. It is possible that withdrawal is associated with decreased functioning in this area, possibly due to diminished dopaminergic tone. Recent studies suggest that nicotine has a facilitatory effect on the dopaminergic response to salient stimuli (Rice and Cragg, 2004; Zhang and Sulzer, 2004). Accordingly, higher plasma levels of nicotine in the non-abstinent smokers could also explain the greater neural response to smoking cues in the non-abstinent state.

The OFC is a key neural substrate in the processing of salient stimuli, as it integrates specific features of reward and determines the proper response based on current circumstances (Breiter et al., 2001; Rolls, 2000). In the present study, we observed seemingly conflicting involvement of the mOFC in response to smoking cues. Whereas the left mOFC was activated during expectancy to smoke, activation of the right mOFC was significantly greater during a state of non-expectancy. Moreover, we showed that neural activity in certain regions was significantly greater for non-expectant subjects than for those expecting to smoke. These findings seem to contradict previous

observations of greater OFC involvement during a state of reward anticipation (Breiter et al., 2001; Knutson et al., 2001), though it is possible that methodological differences account for this incongruity. Interestingly, a possible explanation for this apparent discrepancy comes from a study by Nobre et al. (1999), in which it was shown that breaches of expectancy selectively activated the OFC. This could reflect a state of reward learning in which the presentation of a novel and conflicting reward contingency requires the processing of information related to the situation. However, as we experienced significant artifact-driven BOLD signal loss in the OFC areas reported here, caution should be taken in the interpretation of these findings.

Consistent with their role in the processing of emotional stimuli, the activation of the insula (Damasio, 1999) and mPFC (Simpson et al., 2000) in response to smoking cues seen here could represent the emotional value that these previously neutral stimuli have acquired as a result of continuous pairing with nicotine and its pleasurable effects. However, whether the insula, where activation has been consistently reported in many previous studies of cue-induced drug craving (Garavan et al., 2000; Bonson et al., 2002; Sell et al., 2000; Brody et al., 2002), is involved in the underlying automatic processing of emotional stimuli or the actual conscious perception of craving itself (Bonson et al., 2002) is presently unclear and requires further study. In addition, mPFC activity could also reflect subjects' monitoring of their own emotional reaction to the salient cues, as this region has been implicated in stimuli-induced self-referential thinking (Gusnard et al., 2001). Other regions, such as the precuneus and posterior cingulate, have also been implicated in cue-induced craving (Tapert et al., 2003) and may be involved in the conscious recall of drug-related experiences.

A surprising discovery of this study involved the strong and bilateral activation of a distributed neural network in response to the neutral videos (N > S). Activation of these regions (see Table 2) was consistent throughout all sessions, regardless of expectancy or abstinence state, and may be a consequence of observing tool manipulations during the neutral (haircut) videos. This interpretation is consistent with that of a previous imaging study which showed that observation of action, including the usage of tools, was sufficient to activate a similar network as seen here (Buccino et al., 2001). Although this result is likely unrelated to the response to conditioned cues, it serves to highlight the challenge of selecting appropriate control tasks in cognitive studies.

An unexpected set of findings from this study related to the subjective self-reports of craving, and their interaction with expectancy, abstinence and the BOLD data. For example, we found no distinction between self-reports of positive and negative craving in association with various states of expectancy or abstinence. This would seem to refute our hypothesis stating that positive (appetitive) craving would be associated with a state of expectancy and non-abstinence and that negative (aversive) craving would be more evident during non-expectant and abstinent states. In addition, the finding of greater craving during a non-expectant state than an expectant state was unexpected. However, this higher level of craving was not cue-dependent and so does not challenge previous studies showing that a state of expectancy is associated with greater cue-induced craving (Droungas, 1995; Julian and Brandon, 1998). The reason for these discrepancies was possibly due to the limited sensitivity of the craving questionnaire in the novel scanning environment. The stress of the scanner (i.e. noise, enclosed space, etc.) may have

resulted in the questions being interpreted in a primarily negative manner, an effect that would have been exacerbated during the non-expectant and abstinent states. Accordingly, self-reports of various indices of craving during these states were both greater and independent of the effect of the cue, indicating that abstinent and non-expectant conditions are associated with a primarily unconditioned and aversive craving response.

Discrepancies between the craving and neural responses to conditioned cues were also evident, and these challenge the interpretation of subjective reports in brain imaging studies. Abstinence from smoking, in addition to inducing a definite nicotine withdrawal syndrome, was associated with higher levels of both baseline and cue-induced craving than a non-abstinent state. However, the BOLD response to smoking cues during abstinence was greatly diminished. In keeping with cognitive theories of cue-induced craving (Tiffany, 1990), this apparent incongruity may reveal a fundamental difference between the two neural and subjective responses to drug cues. According to this theory, craving reports are active, non-automatic interpretations of an internal state whereas most neural responses to stimuli are generated by more automatic processes. The possibility that the abstinent and non-expectant states were characterized by reports of negative, unconditioned craving could explain this mismatch. This discrepancy could also explain why we found only a single positive correlation between regions activated by smoking cues (in the left DL-PFC) and the elicited craving, and only in the expectant state. This indicates that the neural activity that results from exposure to conditioned cues may be largely independent from the craving that is concomitantly reported. However, as the BOLD response cannot be considered as an indicator for all possible neural responses,

our results can not definitively establish such dissociation. In addition, as many other studies have found positive links between cue-induced brain activity and craving, it would seem that there is also a significant methodological basis for this incongruity.

CONCLUSIONS AND FUTURE DIRECTIONS

A formidable problem in the study of drug addiction is the determination of how complex human behaviour interacts with the multitude of changes that occur in the addict's brain as a result of chronic drug use. In this regard, the drug user's perception of drug availability reflects a cognitive judgement rooted in normal goal-driven planning processes that are made pathological by a hypersensitive and skewed motivational system. As drug-seeking behaviour is likely always associated with some degree of expectation to use, the full understanding of this process may be necessary for the development of successful addiction treatment. Contemporary theories place the prefrontal cortex at the core of the neural network responsible for guiding behaviour based on complex contextual factors. Furthermore, it is the capacity of the prefrontal cortex to transmit these intentions to actions that may make it a useful target for treatment. Perhaps by learning how to disrupt the processes involved in the anticipation of drug use, drug addicts could avoid the cue-induced reflex of drug seeking and consumption. Indeed, the results of the present study seem to suggest that by being in a mind state of non-expectancy, the addict's conditioned neural response to drug cues can be inhibited. Future studies will be necessary to explore the neural systems involved in how expectancy influences actual drug seeking behaviour. For example, in this study, smokers were exposed to cues that were not actually predictive of drug use and so, a

realistic measure of how drug cues can actually induce expectancy could not be formulated. By modifying the study design, future studies may build on the results shown here to further define the mechanisms by which expectancy modulates the response to drug cues. In one sense, future studies will likely benefit from employing a similar experimental paradigm as used here. By using cigarette smokers, we had the means to modulate both the subject's state of expectancy and abstinence that would not be ethically possible with other drugs of abuse.

The evidence for a disruption of normal responding to salient stimuli seen in this study has definite implications for our conception of addiction. This would seem to imply that during acute withdrawal from a drug, addicts are actually less susceptible to cue-induced craving and drug seeking. Interestingly, the anhedonia experienced during withdrawal may have a similar underlying basis. However, it is probable that, like anhedonia, any such disturbance of the motivation system is temporary, as the danger of cue-induced relapse is very real in the long term. This remains one of the greatest obstacles to treatment in drug addiction, as the aberrant learning processes that take place during chronic drug use lead to very firmly entrenched behaviours in the addict. However, despite this challenge, the ability to combine the experimental cue reactivity paradigm and brain imaging techniques in humans provides an excellent tool for both discovering the underlying mechanisms of drug addiction and testing the efficacy of potential treatment methods.

Another important result from this study was the finding of dissociation between the smokers' subjective response to smoking stimuli and their neural response to the cues. For example, we found that smoking cue-associated craving reports were highest during

an abstinent and non-expectant state, though it was in this state that there was an actual absence of smoking cue-associated fMRI response. This suggests that one should take caution in the interpretation of subjective, emotional reports and how they relate to the actual underlying brain processes. Whether this is due to methodological issues, or whether there is an actual discrepancy between the magnitude of a subjective report and coincident neural activity can not be conclusively ascertained from the data. However, in the present study, it is possible that the type of craving reported between conditions of maximal and minimal cue-induced neural activity (i.e. expectant, non-abstinent vs. non-expectant, abstinent) was fundamentally different and therefore not actually reflective of a dissociation of subjective and neural responses to conditioned stimuli.

In conclusion, this thesis demonstrated directly, for the first time, that the neural response to conditioned drug cues is greatly dependent on both the user's level of expectancy and degree of withdrawal. More specifically, the results showed that this response was augmented by a state of expectancy and diminished during withdrawal. These findings are important in that they increase our knowledge of the role of contextual factors in addiction, and will hopefully contribute to successful treatment strategies in the future.

REFERENCES

Abrams DB, Monti PM, Carey KB, Pinto RP, Jacobus SI (1988) Reactivity to smoking cues and relapse: two studies of discriminant validity. Behav Res Ther 26:225-233.

Alkondon M, Pereira EF, Almeida LE, Randall WR, Albuquerque EX (2000) Nicotine at concentrations found in cigarette smokers activates and desensitizes nicotinic acetylcholine receptors in CA1 interneurons of rat hippocampus. Neuropharmacology 39:2726-39.

Altman J, Everitt BJ, Glautier S, Markou A, Nutt D, Oretti R, Phillips GD, Robbins TW (1996) The biological, social and clinical bases of drug addiction: commentary and debate. Psychopharmacology (Berl) 125:285-345.

Anton D (1999) What is craving? Models and implications for treatment. Alcohol Res Health 23:165-73.

Baker TB, Morse E, Sherman, JE (1987) The motivation to use drugs: psychobiological analysis of urges, in: C. Rivers (Ed.). The Nebraska Symposium on Motivation: alcohol use and abuse, 257-323. Lincoln, University of Nebraska Press.

Balfour DJ, Wright AE, Benwell ME, Birrell CE (2000) The putative role of extra-

synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. Behav Brain Res 113:73-83.

Balfour DJ (2002) The neurobiology of tobacco dependence: a commentary. Respiration 69(1):7-11.

Benowitz NL (1999) The biology of nicotine dependence: from the 1988 Surgeon General's Report to the present and into the future. Nicotine Tob Res 1:S159-63.

Berger SP, Hall S, Mickalian JD, Reid MS, Crawford CA, Delucchi K, Carr K, Hall S (1996) Haloperidol antagonism of cue-elicited cocaine craving. Lancet 347:504-8.

Bonson KR, Grant SJ, Contoreggi CS, Links JM, Metcalfe J, Weyl HL, Kurian V, Ernst M, London ED (2002) Neural systems and cue-induced cocaine craving. Neuropsychopharmacology 26:376-386.

Brass M, Zysset S, von Cramon DY (2001) The inhibition of imitative response tendencies. Neuroimage 14:1416-23.

Breiter HC, Aharon I, Kahneman D, Dale A, Shizgal P (2001) Functional imaging of neural responses to expectancy and experience of monetary gains and losses. Neuron 30:619-639.

Brody AL, Mandelkern MA, London ED, Childress AR, Lee GS, Bota RG, Ho ML, Saxena S, Baxter LR Jr, Madsen D, Jarvik ME (2002) Brain metabolic changes during cigarette craving. Arch Gen Psychiatry 59:1162-1172.

Brody AL, Mandelkern MA, Lee G, Smith E, Sadeghi M, Saxena S, Jarvik ME, London ED (2004) Attenuation of cue-induced cigarette craving and anterior cingulate cortex activation in bupropion-treated smokers: a preliminary study. Psychiatry Res 130:269-81.

Buccino G, Binkofski F, Fink GR, Fadiga L, Fogassi L, Gallese V, Seitz RJ, Zilles K, Rizzolatti G, Freund HJ (2001) Action observation activates premotor and parietal areas in a somatotopic manner: an fMRI study. Eur J Neurosci 13:400-404.

Carter CS, Botvinick MM, Cohen JD (1999) The contribution of the anterior cingulate cortex to executive processes in cognition. Rev Neurosci 10:49-57.

Carter BL, Tiffany ST (1999) Meta-analysis of cue-reactivity in addiction research. Addiction 94:327-40.

Carter BL, Tiffany ST (2001) The cue-availability paradigm: the effects of cigarette availability on cue reactivity in smokers. Exp Clin Psychopharmacol 9:183-90.

Childress AR, McLellan AT, O'Brien CP (1986) Abstinent opiate abusers exhibit conditioned craving, conditioned withdrawal and reductions in both through extinction. Br J Addict 81:655-60.

Childress AR, McLellan AT, Ehrman R, O'Brien CP (1988) Classically conditioned responses in opioid and cocaine dependence: a role in relapse? NIDA Res Monogr 84:25-43.

Childress AR, Hole AV, Ehrman RN, Robbins SJ, McLellan AT, O'Brien CP (1993) Cue reactivity and cue reactivity interventions in drug dependence. NIDA Res Monogr 137:73-95.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. Am J Psychiatry 156:11-18.

Ciccocioppo R, Sanna PP, Weiss F (2001) Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D1 antagonists. Proc Natl Acad Sci USA 98:1976-81.

Cloninger CR, Przybeck TR, Svrakic DM (1991) The tridimensional personality questionnaire: U.S. normative data. Psychol. Rev 69:1047–1057.

Collins DL, Neelin P, Peters TM, Evans AC (1994) Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr 18:192-205.

Critchley HD, Mathias C J, Josephs O, O'Doherty J, Zanini S, Dewar BK, Cipolotti L, Shallice T, Dolan RJ (2003) Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. Brain 126:2139-52.

Daglish MR, Weinstein A, Malizia AL, Wilson S, Melichar JK, Britten S, Brewer C, Lingford-Hughes A, Myles JS, Grasby P, Nutt DJ (2001) Changes in regional cerebral blood flow elicited by craving memories in abstinent opiate-dependent subjects. Am J Psychiatry 158:1680-1686.

Damasio A (1999) The Feeling of What Happens: Body and Emotion in the Making of Consciousness. New York, Harcourt Brace & Company.

Dehaene S, Changeux JP (2000) Reward-dependent learning in neuronal networks for planning and decision making. Prog Brain Res 126:217-29.

Deroche V, Le Moal M, Piazza PV (2004) Cocaine self-administration increases the incentive motivational properties of the drug in rats. Eur J Neurosci 11:2731-6.

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85:5274-8.

Domino EF, Minoshima S, Guthrie SK, Ohl L, Ni L, Koeppe RA, Cross DJ, Zubieta J (2000a) Effects of nicotine on regional cerebral glucose metabolism in awake resting tobacco smokers. Neuroscience 101:277-82.

Domino EF, Minoshima S, Guthrie S, Ohl L, Ni L, Koeppe RA, Zubieta JK (2000b) Nicotine effects on regional cerebral blood flow in awake, resting tobacco smokers. Synapse 38:313-21.

Donny EC, Caggiula AR, Mielke MM, Booth S, Gharib MA, Hoffman A, Maldovan V, Shupenko C, McCallum SE (1999) Nicotine self-administration in rats on a progressive ratio schedule of reinforcement. Psychopharmacology (Berl) 147:135-42.

Droungas A, Ehrman RN, Childress AR, O'Brien CP (1995) Effect of smoking cues and cigarette availability on craving and smoking behavior. Addict Behav 20:657-673.

Drummond S, Tiffany ST, Glautier S, Remington B (1995) Cue exposure in understanding and treating addictive behaviours, in Drummond S, Tiffany ST, Glautier S, Remington B (Eds), Addictive Behaviours: cue exposure theory and practice, 1-17. Chichester, John Wiley & Sons.

Due DL, Huettel SA, Hall WG, Rubin DC (2002) Activation in mesolimbic and visuospatial neural circuits elicited by smoking cues: evidence from functional magnetic resonance imaging. Am J Psychiatry 159:954-960.

Eichhammer P, Johann M, Kharraz A, Binder H, Pittrow D, Wodarz N, Hajak G (2003) High-frequency repetitive transcranial magnetic stimulation decreases cigarette smoking. J Clin Psychiatry 64:951-953.

Epping-Jordan MP, Watkins SS, Koob GF, Markou A (1998) Dramatic decreases in brain reward function during nicotine withdrawal. Nature 393:76-79.

Field M, Duka T (2001) Smoking expectancy mediates the conditioned responses to arbitrary smoking cues. Behav Pharmacol 12:183-94.

Fletcher PJ, Korth KM, Sabijan MS, DeSousa NJ (1998) Injections of D-amphetamine into the ventral pallidum increase locomotor activity and responding for conditioned reward: a comparison with injections into the nucleus accumbens. Brain Res 805:29-40.

Fontana DJ, Post RM, Pert A (1993) Conditioned increases in mesolimbic dopamine overflow by stimuli associated with cocaine. Brain Res 629:31-9.

Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA (2000) Cue-induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. Am J Psychiatry 157:1789-1798.

George MS, Anton RF, Bloomer C, Teneback C, Drobes DJ, Lorberbaum JP, Nahas Z, Vincent DJ (2001) Activation of prefrontal cortex and anterior thalamus in alcoholic subjects on exposure to alcohol-specific cues. Arch Gen Psychiatry 58:345-52.

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642-52.

Gong W, Neill D, Justice JB Jr. (1997) 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. Brain Res 754:103-112

Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C, Phillips RL, Kimes AS, Margolin A (1996) Activation of memory circuits during cue-elicited cocaine craving. Proc Natl Acad Sci USA 15:12040-12045.

Gross TM, Jarvik ME, Rosenblatt MR (1993) Nicotine abstinence produces content-specific Stroop interference. Psychopharmacology (Berl) 110:333-336.

Gusnard DA, Akbudak E, Shulman GL, Raichle ME (2001) Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc Natl Acad Sci USA 98:4259-4264.

Haney M, Foltin RW, Fischman MW (1998) Effects of pergolide on intravenous cocaine self-administration in men and women. Psychopharmacology (Berl) 137:15-24.

Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO (1991) The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict 86:1119-1127.

Heeger DJ, Ress D (2002) What does fMRI tell us about neuronal activity? Nat Rev Neurosci 3:142-51.

Hildebrand BE, Nomikos GG, Hertel P, Schilstrom B, Svensson TH (1998) Reduced dopamine output in the nucleus accumbens but not in the medial prefrontal cortex in rats displaying a mecamylamine-precipitated nicotine withdrawal syndrome. Brain Res 779:214-25.

Isomura Y, Ito Y, Akazawa T, Nambu A, Takada M (2003) Neural coding of "attention for action" and "response selection" in primate anterior cingulate cortex. J Neurosci 23:8002-12.

Johnsen BH, Thayer JF, Laberg JC, Asbjornsen AE (1997) Attentional bias in active smokers, abstinent smokers, and nonsmokers. Addict Behav 22:813-817.

Juliano LM, Brandon TH (2002) Effects of nicotine dose, instructional set, and outcome expectancies on the subjective effects of smoking in the presence of a stressor. J Abnorm Psychol 111:88-97.

Kerns JG, Cohen JD, MacDonald AW 3rd, Cho RY, Stenger VA, Carter CS (2004) Anterior cingulate conflict monitoring and adjustments in control. Science 303:1023-1026.

Klitenick MA, Deutch AY, Churchill L, Kalivas PW (1992) Topography and functional role of dopaminergic projections from the ventral mesencephalic tegmentum to the ventral pallidum. Neuroscience 50:371-386.

Koob GF, Le Moal M (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52-8.

Knutson B, Fong GW, Adams CM, Varner JL, Hommer D (2001) Dissociation of reward anticipation and outcome with event-related fMRI. Neuroreport 12:3683-3687.

Krawczyk DC 2002 Contributions of the prefrontal cortex to the neural basis of human decision making. Neurosci Biobehav Rev 26:631-64.

LeDoux JE (2000) Emotion circuits in the brain. Annu Rev Neurosci 23:155-84.

Le Foll B, Diaz J, Sokoloff P (2002) Increased dopamine D3 receptor expression accompanying behavioral sensitization to nicotine in rats. Synapse 47:176-83.

Logothetis NK, Wandell BA (2004) Interpreting the BOLD signal. Annu Rev Physiol 66:735-69.

London ED, Ernst M, Grant S, Bonson K, Weinstein A (2000) Orbitofrontal cortex and human drug abuse: functional imaging. Cereb Cortex 10:334-42.

London ED, Simon SL, Berman SM, Mandelkern MA, Lichtman AM, Bramen J, Shinn AK, Miotto K, Learn J, Dong Y, Matochik JA, Kurian V, Newton T, Woods R, Rawson R, Ling W (2004) Mood disturbances and regional cerebral metabolic abnormalities in recently abstinent methamphetamine abusers. Arch Gen Psychiatry 61:73-84.

Markou A, Weiss F, Gold LH, Caine SB, Schulteis G, Koob GF (1993) Animal models of drug craving. Psychopharmacology (Berl) 112:163-82.

McGehee DS, Heath MJ, Gelber S, Devay P, Role LW (1995) Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. Science 269:1692-6.

McNair D, Lorr M, Droppleman L (1992) EITS Manual for Profile of Mood States. San Diego.

Meil WM, See RE (1997) Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. Behav Brain Res 87:139-48.

Miller EK (2000) The prefrontal cortex and cognitive control. Nat Rev Neurosci 1:59-65.

Miyata H, Yanagita T (2001) Neurobiological mechanisms of nicotine craving. Alcohol 24:87-93.

Modell JG, Mountz JM (1995) Focal cerebral blood flow change during craving for alcohol measured by SPECT. J Neuropsychiatry Clin Neurosci 7:15-22.

Monchi O, Petrides M, Doyon J, Postuma RB, Worsley K, Dagher A (2004) Neural bases of set-shifting deficits in Parkinson's disease. J Neurosci 24:702-710.

Morgan MJ, Davies GM, Willner P (1999) The Questionnaire of Smoking Urges is sensitive to abstinence and exposure to smoking-related cues. Behav Pharmacol 10:619-26.

Nisell M, Nomikos GG, Svensson TH (1994) Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse 16:36-44.

Nobre AC, Coull JT, Frith CD, Mesulam MM (1999) Orbitofrontal cortex is activated during breaches of expectation in tasks of visual attention. Nat Neurosci 2:11-2.

O'Doherty JP, Deichmann R, Critchley HD, Dolan RJ (2002) Neural responses during anticipation of a primary taste reward. Neuron 33(5):815-826.

Ongur D, Ferry AT, Price JL (2003) Architectonic subdivision of the human orbital and medial prefrontal cortex. J Comp Neurol 460:425-49.

Paus T (2000) Functional anatomy of arousal and attention systems in the human brain. Prog Brain Res 126:65-77.

Paus T (2001) Primate anterior cingulate cortex: where motor control, drive and cognition interface. Nat Rev Neurosci 2:417-424.

Petrides M, Pandya DN (1999) Dorsolateral prefrontal cortex: comparative cytoarchitectonic analysis in the human and the macaque brain and corticocortical connection patterns. Eur J Neurosci 11:1011-36.

Petrides M (2000) The role of the mid-dorsolateral prefrontal cortex in working memory. Exp Brain Res 133:44-54.

Phelps ME (2000) PET: the merging of biology and imaging into molecular imaging. J Nucl Med 41:661-81.

Phillips ML, Drevets WC, Rauch SL, Lane R (2003) Neurobiology of emotion perception I: The neural basis of normal emotion perception. Biol Psychiatry 54:504-14.

Powell J, Tait S, Lessiter J (2002) Cigarette smoking and attention to signals of reward and threat in the Stroop paradigm. Addiction 97:1163-1170.

Price JL (2003) Comparative aspects of amygdala connectivity. Ann N Y Acad Sci 985:50-8.

Quirk GJ, Gehlert DR (2003) Inhibition of the amygdala: key to pathological states? Ann N Y Acad Sci 985:263-72.

Rice ME, Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. Nat Neurosci 7:583-584.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-91.

Roesch MR, Olson CR (2003) Impact of expected reward on neuronal activity in prefrontal cortex, frontal and supplementary eye fields and premotor cortex. J Neurophysiol 90:1766-89.

Rohsenow D J, M onti P M, R ubonis A V, S irota AD, N iaura R S, C olby SM, W unschel SM, Abrams DB (1994) Cue reactivity as a predictor of drinking among male alcoholics. J Consult Clin Psychol 62:620-6.

Rolls ET, Sienkiewicz ZJ, Yaxley S (1989) Hunger modulates the responses to gustatory stimuli of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. Eur J Neurosci 1:53-60.

Rolls ET (2000) The orbitofrontal cortex and reward. Cereb Cortex 10:284-294

Rose JE, Behm FM, Westman EC, Mathew RJ, London ED, Hawk TC, Turkington TG, Coleman RE (2003) PET studies of the influences of nicotine on neural systems in cigarette smokers. Am J Psychiatry 160:323-33.

Rusted JM, Caulfield D, King L, Goode A (2000) Moving out of the laboratory: does nicotine improve everyday attention? Behav Pharmacol 11:621-9.

Sayette M A, S hiffman S, T iffany S T, Niaura R S, M artin C S, S hadel WG (2000) T he measurement of drug craving. Addiction 2:S189-210.

Schaefer GJ, Michael RP (1986) Task-specific effects of nicotine in rats. Intracranial self-stimulation and locomotor activity. Neuropharmacology 25:125-31.

Schoffelmeer AN, De Vries TJ, Wardeh G, van de Ven HW, Vanderschuren LJ (2002) Psychostimulant-induced behavioral sensitization depends on nicotinic receptor activation. J Neurosci 22:3269-76.

Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1-27.

Sell LA, Morris JS, Bearn J, Frackowiak RS, Friston KJ, Dolan RJ (2000) Neural responses associated with cue evoked emotional states and heroin in opiate addicts. Drug Alcohol Depend 60:207-216.

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl) 168:3-20.

Shiffman SM, Jarvik ME (1976) Smoking withdrawal symptoms in two weeks of abstinence. Psychopharmacology (Berl) 50:35-39.

Siegel S (1983) Classical conditioning, drug tolerance, and drug dependence. In R.G. Smart, F.B. Glaser, Y. Israel, et al. (Eds.), Research advances in alcohol and drug problems 7:207-246. New York: Plenum.

Simpson JR, Ongur D, Akbudak E, Conturo TE, Ollinger JM, Snyder AZ, Gusnard DA, Raichle ME (2000) The emotional modulation of cognitive processing: an fMRI study. J Cogn Neurosci Suppl 2:157-70.

Solomon RL, Corbit JD (1973) An opponent-process theory of motivation. II. Cigarette addiction. J Abnorm Psychol 81:158-71.

Spanagel R, Weiss F (1999) The dopamine hypothesis of reward: past and current status. Trends Neurosci 22:521-527.

Stein EA, Pankiewicz J, Harsch HH, Cho JK, Fuller SA, Hoffmann RG, Hawkins M, Rao SM, Bandettini PA, Bloom AS (1998) Nicotine-induced limbic cortical activation in the human brain: a functional MRI study. Am J Psychiatry 155:1009-15.

Stewart J, de Wit H, Eikelboom R (1984) Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 91:251-68.

Stolerman IP, Shoaib M (1991) The neurobiology of tobacco addiction. Trends Pharmacol Sci 12:467-73.

Tapert SF, Cheung EH, Brown GG, Frank LR, Paulus MP, Schweinsburg AD, Meloy MJ, Brown SA (2003) Neural response to alcohol stimuli in adolescents with alcohol use disorder. Arch Gen Psychiatry 60:727-735.

Tiffany ST (1990) A cognitive model of drug urges and drug-use behavior: role of automatic and nonautomatic processes. Psychol Rev 97:147-68.

Tiffany ST, Drobes DJ (1991) The development and initial validation of a questionnaire on smoking urges. Br J Addict 86:1467-1476.

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642-1652.

van Dyck CH, Rosen MI, Thomas HM, McMahon TJ, Wallace EA, O'Connor PG, Sullivan M, Krystal JH, Hoffer PB, Woods SW (1994) SPECT regional cerebral blood flow alterations in naltrexone-precipitated withdrawal from buprenorphine. Psychiatry Res 55:181-191.

Vastola BJ, Douglas LA, Varlinskaya EI, Spear LP (2002) Nicotine-induced conditioned place preference in adolescent and adult rats. Physiol Behav 77:107-14.

Vidal C, Changeux JP (1993) Nicotinic and muscarinic modulations of excitatory synaptic transmission in the rat prefrontal cortex in vitro. Neuroscience 56:23-32.

Volkow ND, Fowler JS, Wolf AP, Hitzemann R, Dewey S, Bendriem B, Alpert R, Hoff A (1991) Changes in brain glucose metabolism in cocaine dependence and withdrawal. Am J Psychiatry 148:621-6.

Volkow ND, Fowler JS, Wang GJ, Hitzemann R, Logan J, Schlyer DJ, Dewey SL, Wolf AP (1993) Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. Synapse 14:169-77.

Volkow ND, Logan J, Fowler JS, Wang GJ, Gur RC, Wong C, Felder C, Gatley SJ, Ding YS, Hitzemann R, Pappas N (2000) Association between age-related decline in brain dopamine activity and impairment in frontal and cingulate metabolism. Am J Psychiatry 157:75-80.

Volkow ND, Wang GJ, Ma Y, Fowler JS, Zhu W, Maynard L, Telang F, Vaska P, Ding YS, Wong C, Swanson JM (2003) Expectation enhances the regional brain metabolic and the reinforcing effects of stimulants in cocaine abusers. J Neurosci 23:11461-11468.

West R, Gossop M (1994) Overview: a comparison of withdrawal symptoms from different drug classes. Addiction 89:1483-9.

Wexler B E, Gottschalk C H, Fulbright R K, Prohovnik I, Lacadie C M, Rounsaville B J, Gore JC (2001) Functional magnetic resonance imaging of cocaine craving. Am J Psychiatry 158:86-95.

Wikler A (1948) Recent progress in research on the neurophysiological basis of morphine addiction. Am J Psychiatry 105:329-338.

Williams JM, Mathews A, MacLeod C (1996) The emotional Stroop task and psychopathology. Psychol Bull 120:3-24.

Willner, P, Hardman, S, Eaton, G (1995). Subjective and behavioural evaluation of cigarette cravings. Psychopharmacology 118:171-177.

Wilson SJ, Sayette MA, Fiez JA (2004) Prefrontal responses to drug cues: a neurocognitive analysis. Nat Neurosci 7:211-214.

Worsley KJ, Liao CH, Aston J, Petre V, Duncan GH, Morales F, Evans AC (2002) A general statistical analysis for fMRI data. Neuroimage 15:1-15.

Wu JC, Bell K, Najafi A, Widmark C, Keator D, Tang C, Klein E, Bunney BG, Fallon J, Bunney WE (1997) Decreasing striatal 6-FDOPA uptake with increasing duration of cocaine withdrawal. Neuropsychopharmacology 17:402-409.

Zhang H, Sulzer D (2004) Frequency-dependent modulation of dopamine release by nicotine. Nat Neurosci 7:581-582

APPENDIX

Ethics Certificate

See attached.

Centre universitaire de santé McGill
McGill University Health Centre

12 May 2003

Dr Alain Dagher
McConnell Brain Imaging Centre
Montreal Neurological Institute

3.i. DAGA 2002/1 **Regional Brain Activation Associated with Cue-induced Nicotine Craving (CIHR MOP 49480)**
Continuing Review checklist of 2003.04.14, English and French consent documents of 2002.03.23

We have received an Application for Continuing Review for the research study as above. The report was presented for full board review at the convened meeting of the MNI/H Research Ethics Board (REB) on 2003.04.28, was found to be acceptable for continuation at the McGill University Health Centre (MUHC) and this has accordingly been entered into the minutes of the meeting.

The reapproval of the study is valid until 2004.04.21. The REB noted that no revision to the approved consent documents as above is required at this time.

All research involving human subjects requires review at recurring intervals. To comply with the regulation for continuing review of "at least once per year," it is the responsibility of the investigator to submit an Application for Continuing Review to the REB prior to expiry.

However, should the research conclude for any reason prior to approval expiry, you are required to submit a Termination Report to the board once the data analysis is complete to give an account of the study findings and publication status.

Should any revision to the study or other development occur prior to the next required review, you must advise the REB without delay. Regulation does not permit initiation of a proposed study modification prior to REB approval of the amendment.

We trust this will prove satisfactory to you.

Yours very truly,

Co - Chair

Eugene Bereza, MD CM, CCFP, Chair
MNH/I Research Ethics Board
/ve

Cc: MUHC Study #DAGA 2002/1