Motivational mechanisms underlying General Pavlovian-to-Instrumental Transfer (PIT): The effects of negative mood

By

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Declaration

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

Signature . . . . . .

Date . . . . . .

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Contents

List of Abbreviations

Acute tryptophan depletion: ATD

Affective go/no-go: AGNG

Beck Depression Inventory: BDI

Brown Norway: BN

Conditioned response: CR

Conditioned stimulus: CS

Conditioned place preference: CPP

Cued-reinforcement reaction-time task: CRRT

Mood Induction Procedure: MIP

One touch tower of London: OTT

Outcome-response: O-R

Pavlovian-to-Instrumental transfer: PIT

Profile of Mood States: POMS

Response-outcome: R-O

Self-referent encoding/retrieval task: SRET

Serotonin transporter: 5-HTTLPR

Serotonin: 5-HT

Sprague Dawley: SD

Stimulus-outcome: S-O

Stimulus-response: S-R

Stop signal task: SST

Unconditioned response: UR

Unconditioned stimulus: US

Visual analogue scale: VAS

Preface

This thesis is written in the European/US format, whereby a collection of studies are presented as manuscripts prepared for publication. The manuscripts are preceded by an overview chapter and a general conclusions chapter. The manuscripts represent my own work (with supervisory input from the additional authors). I wrote the first draft and took the lead on all subsequent revisions.

Chapter 3 is in preparation as:

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> Mathers, C., Steckler, T., & Duka, T. (2014). The impact of positive and negative mood on the speed and accuracy of acquiring Pavlovian contingencies of stimuli predictive of reward.

Thesis Summary

The extent to which motivational mechanisms contribute to reward seeking processes is crucial to our understanding of certain abnormal behaviours, including addiction. Pavlovian conditioning endows reward-associated stimuli with the ability to modulate goal-directed actions for that same reward (Pavlovian-to-Instrumental transfer; PIT). Learning and motivational theories attempt to describe the processes by which stimuli in the environment acquire incentive properties, attract attention and drive reward-seeking behaviours and bear many resemblances, but there are also important differences. This thesis uses a general PIT model in humans to further our understanding of these discrepancies and investigates the effect mood has on these processes.

Firstly, altering the value of the reward affected the rigor of instrumental performance, but the same changes in outcome value did not affect the expectancy of, attention to, or emotional reactivity to the cues suggesting that in Pavlovian learning, apart from the nature of outcomes, the value of outcomes is encoded such that changes in outcome value prevent transfer of a Pavlovian cue's incentive properties to alter goal-directed action. Secondly, the further papers assess the extent to which mood modulates this same action. When under negative mood a general reduction in motivation, driven by an attenuated sensitivity to the reward was observed, as well as a dissociation between aversive and appetitive outcomes. The remaining study explored whether mood altered Pavlovian learning and revealed that those under state negative mood take longer to express their knowledge explicitly and that those under positive mood showed altered attention and emotional responses towards the same stimuli.

The approach used in this thesis shows the merits of both motivational and learning theories, and further demonstrates the link between mood and motivation. Additionally,

a dissociation between punishment and reward prediction when under negative mood was demonstrated and builds upon this important distinction.

Chapter 1

General Introduction

Depressive disorders, drug addiction and classical conditioning

Depressive disorders are often co-morbid with anxiety disorders, which represent the most common type of mental disorder across a range of countries (Demyttenaere et al., 2004), but patients do not always receive the appropriate attention for their condition when compared to other mental illnesses (Kroenke, Spitzer, Williams, Monahan, & Lowe, 2007). Anxiety and depression often present with heavy alcohol use, addiction issues and smoking (Martin-Merino, Ruigomez, Johansson, Wallander, & Garcia-Rodriguez, 2010) and the main focus of this thesis will surround the relationship between depressive mood and addiction; in particular the impact current mood has on motivation. This link is important as a key symptom of depression is anhedonia (Huprich, 2013). Anhedonia is associated with decreased sensitivity to pleasurable events (Der-Avakian & Markou, 2012), which may be modelled experimentally using reward outcomes in a Pavlovian design. As such, mood may therefore modulate the motivated responses to rewards, and this will be discussed in more detail later. Furthermore, animal studies have demonstrated the presence of anhedonia by adopting the use of procedures that are sensitive to experimental manipulations in reward value. Accordingly, subjects with negative mood should not be sensitive to the effects of increased or decreased reward value in the expression of conditioning and investigating this is important in furthering our understanding of the link between depression and reward which is another focus of this thesis. It is also important in supporting the development of therapeutic interventions for addiction.

Main model of classical conditioning

Classical conditioning processes can render cues signalling reward and aversive outcomes with the ability to initiate affective responses. A neutral stimulus can be repeatedly and reliably paired with an unconditioned stimulus (US) such that eventually the neutral stimulus becomes conditioned (conditioned stimulus; CS) and able to generate a response that sometimes mimics the response generated by the US (unconditioned response; UR). The mimicked response is known as the conditioned response (CR) and is the outcome of successful classical conditioning (Rescorla, 1967).

Classical conditioning is dependent upon several key principles, such as expectation and contingency. It is extremely important that the US is more likely to occur after presentation of the CS than in the absence of the CS, i.e. they are contingent upon each other (Rescorla, 1968). A further requirement is that an element of surprise must be associated with the US to elicit successful conditioning (Rescorla, 1976).

Conditioned appetitive responses and activation of an appetitive motivational system

Stimuli associated with a reward outcome may eventually come to drive the same response generated by the reward itself and this is known as a conditioned appetitive response. Animal and human drug addiction literature is abundant with examples of how stimuli paired with a reward can elicit an appetitive response. In animals this has been demonstrated using drugs such as opiates (Hand, Stinus, & Le Moal, 1989) in a conditioned place preference (CPP) paradigm, and in humans a stimulus previously associated with cocaine is more often chosen than a stimulus associated with placebo by cocaine abusers (Foltin & Haney, 2000). It is, however, harder to elicit such CRs by humans for non-drug rewards unless the stimuli are particularly arousing, as

demonstrated by the ability of a geometric figure paired with an erotic image to bring about increased ratings of pleasantness when compared to a pairing with a non-erotic image (Klucken et al., 2009). In another example, extreme images (i.e. erotica and violent death) have been shown to bring about responses such as increased skin conductance; as such conditioned emotional responses are also indicative of cue-elicited motivated behaviour (Bindra, 1969), the link between emotion and motivation is an important one. In addition, as highly emotive images are successfully used to induce state mood (Gilet, 2008), as well as induce other behavioural and physiological responses (Lang, Greenwald, Bradley, & Hamm, 1993; Smith, Bradley, & Lang, 2005), this demonstrates the link between state mood, motivation and reward related behaviour. Such physiological responses (i.e. skin conductance as well as increases in heart rate) have also been shown to be brought about by cues such as smoking (Tiffany & Drobes, 1990) and alcohol (Glautier, Drummond, & Remington, 1994).

Furthermore, during conditioning through associative learning mechanisms, links can be formed between representations of stimuli in the environment and reward outcome (Everitt, Cardinal, Parkinson, & Robbins, 2003; Everitt & Robbins, 2005); this is often preceded by an instrumental response (Hogarth, Dickinson, & Duka, 2005; Hogarth, Dickinson, Hutton, Elbers, & Duka, 2006). The action of instrumental responding in the presence of such stimuli associated with reward indicates activation of an appetitive motivational system. Once a predictive associative relationship is established the stimuli become able to control the instrumental response (Hogarth, et al., 2005) acquiring the ability to lead to impulsive reward seeking, (Dalley et al., 2009; Pelloux, Everitt, & Dickinson, 2007; Vanderschuren & Everitt, 2004) sometimes despite negative outcomes (Everitt et al., 2007). As such the stimuli in the environment acquire incentive properties (Everitt & Robbins, 2005), attract attention and drive the reward seeking

behaviour (Hogarth, et al., 2005; Hogarth & Duka, 2006a). In periods of abstinence stimuli can reinstate reward seeking behaviour as demonstrated in animals (e.g. Economidou, Pelloux, Robbins, Dalley, & Everitt, 2009; Pelloux, et al., 2007; Vanderschuren & Everitt, 2004, etc.) and the role of environmental stimuli under such circumstances has also been investigated (Everitt, et al., 2007). The ability of an altered value of rewarded outcome to maintain control of the instrumental response and the effect mood may play on the acquisition of such predictive associative relationships is investigated in this thesis. I will argue that state mood is an important factor in reward seeking, and that this may be utilised in therapeutic interventions for drug addiction.

Main mechanisms underlying reward seeking behaviour

Both learning and motivational theories attempt to describe the underlying effect of the stimuli on reward seeking behaviour. Learning theories suggest that the presence of the stimuli activates mental representations of the outcome and thus drives the behaviour (Hogarth, Dickinson, Austin, Brown, & Duka, 2008; Hogarth, et al., 2005; Hogarth, Dickinson, Hutton, Bamborough, & Duka, 2006; Hogarth, Dickinson, Hutton, Elbers, et al., 2006); in contrast motivational theories suggest the presence of the stimuli increases motivation more generally and thus drives the behaviour to obtain the reward (Everitt et al., 2008). Common causal factors that contribute to the effect of the stimulus as described in both theories include reward value, such that increased reinforcer value increases salience for the stimulus, and deprivation from reward for a period of time increases the behavioural output to seek reward such that deprivation increases the value of the reward (Everitt & Robbins, 2005; Hogarth & Duka, 2006a). This thesis also aims to investigate these differences, and the impact state mood may have. The general link between negative mood and reward has previously been demonstrated (Rogers et al.,

2003), and negative mood has been shown to be involved in many facets involved in learning and motivated behaviour, and this is discussed in detail later.

Pavlovian-to-Instrumental Transfer

As touched upon earlier, increased performance of an instrumental response in the presence of a CS is indicative of the activation of an appetitive motivational system by the CS. It is thought that a central appetitive motivational system could become activated and be responsible for driving the instrumental responding. Alternatively, the response could be related to a specific representation of the reward driving the responding through specific outcome expectancy. One method by which outcome-specific and general motivational systems have been separated is Pavlovian-to-Instrumental transfer (PIT). In summary, however, classical conditioning and instrumental responding are trained independently in PIT, and if as a result of the combination of both training sessions the Pavlovian cue elicits selective responding for the specific reward it was paired with, then an outcome-specific theory is supported. However, if responding is increased more generally, i.e. for different rewards, then a more general motivational system has been activated which can be dissociated from a specific CS-US representation; whilst both have been demonstrated experimentally, the former using monetary and cigarette rewards (Hogarth, Dickinson, Wright, Kouvaraki, & Duka, 2007), the general model is associated with certain drug rewards and has also been carried out successfully in animals utilising cues associated with alcohol which also enhanced responding for non-alcohol reward (Corbit & Janak, 2007).

PIT is a form of associative learning in which conditioned Pavlovian cues enhance instrumental responding for rewards previously associated with those cues and for

others. This occurs even when those rewards are no longer available and in the absence of any explicit training between the Pavlovian and instrumental contingencies (Bray, Rangel, Shimojo, Balleine, & O'Doherty, 2008; Talmi, Seymour, Dayan, & Dolan, 2008). In other words, PIT represents the capacity of conditioned stimuli to augment instrumental behaviour. Since Estes (Gutman & Estes, 1949) first demonstrated the PIT effect in rats, the phenomenon has been reproduced in several animal studies (Colwill & Rescorla, 1990; Lovibond, 1983; Rescorla, 1994a) and more recently with humans (Bray, et al., 2008; Hogarth, et al., 2007; Talmi, et al., 2008). Investigation is now focused upon identifying underlying mechanisms and exploring variations within the PIT paradigm. For example, Trick et al. (Trick, Hogarth, & Duka, 2011) developed the PIT technique to include probabilities of reward; providing a method for quantifying mental representations of outcome-expectancies.

Many behaviours may be influenced by conditioned stimuli associated with certain outcomes (a type of PIT), one of the most significant (in terms of its impact on society, crime, and (mental) health) is drug abuse (Everitt, Dickinson, & Robbins, 2001). Certain contextual cues (e.g. drug paraphernalia, location of consumption, etc.) are thought to become associated with drug taking. When these cues are re-encountered, they motivate drug-seeking and hence drug taking. This can maintain drug-taking and has been documented as a major form of relapse within drug addiction, often after years of abstinence (Glautier, et al., 1994). However, the underlying mechanisms are still subject to debate. Tiffany (Tiffany, 1990; Tiffany & Drobes, 1990) propose this form of drug taking reflects habitual stimulus-response (S-R) links, whereby once the stimulus-response association has been established (by initial pairing with the outcome) the stimulus alone is enough to elicit the CR (drug-seeking), independent of outcome expectancy. Alternatively, the rival hypothesis states that cue-elicited drug taking is a

manifestation of goal-directed behaviour, driven by a mental-representation of the contingency between response and outcome (Holland, 2007; Olmstead, Lafond, Everitt, & Dickinson, 2001). If an S-R habit is formed, devaluation of the outcome should not change the increased response elicited by CSs, because the outcome is irrelevant to the S-R link and the vital S-R association has not been altered. Conversely, goal-directed behaviour would decrease/discontinue with reinforcer devaluation because presumably the diminished goal would not be adequate to motivate behaviour. The PIT paradigm offers the best procedure to study the mechanisms by which stimuli can control reinforcer seeking behaviour (Bray, et al., 2008; Declercq & De Houwer, 2009; Hogarth, et al., 2007; Paredes-Olay, Abad, Gamez, & Rosas, 2002; Talmi, et al., 2008). From the animal literature it becomes apparent that reinforcer transfer is resistant to devaluation (e.g. Holland, 2004; Rescorla, 1994a, etc.) and therefore the behavioural response cannot be driven by the expected incentive value of the outcome. According to Rescorla (1994a) the conditioned stimulus signals sensory aspects of the outcome (S-O associations) learned during Pavlovian training which in turn activates the response (O-R associations) irrespective of the current value of the outcome (i.e. R-O current relationship is of no importance); thus lack of an effect of current devaluation of the reinforcer is consistent with this view (Holland, 2004; Rescorla, 1994b). These observations hold true for reinforcer specific transfer, which differs from transfer in a general paradigm. In the former, specific CSs associated with particular outcomes will come to activate response associated with the outcome they predict but not responses associated with a different outcome. In the general PIT, conditioned stimuli enhance an instrumental response via their emotional significance without directly influencing responding based on the sensory characteristics of the reinforcer as occurs in the outcome specific PIT (Dawson & Dickinson, 1990). Although the strength of the

response in the outcome specific transfer is not affected by the current value of the reinforcer, it appears to be modulated by the strength of the stimulus outcome contingency in training both in animal and humans.

In conclusion PIT is a useful model for investigating many aspects of learning and reward seeking, and also presents a tool whereby effects of altered reinforcer value may be observed. As negative mood is associated with anhedonia and altered perception of reward, PIT is an ideal model for studying associated effects of manipulated state mood that may include, but are not limited to, altered behaviour and response to reward.

General Materials for Pavlovian-to-Instrumental Transfer

The stimuli used will be visual abstract stimuli, which have previously been successfully conditioned to both aversive (Hogarth, Dickinson, et al., 2008) and appetitive outcomes (Hogarth, Dickinson, Janowski, Nikitina, & Duka, 2008). These stimuli will be presented in neutral grey-scale rather than bright colours, as certain colours have been shown to affect mood (Hamid & Newport, 1989) which would hamper future investigations into mood and are shown in the appendix (appendix 1). Additionally, individual differences in current mood state and the presence of emotional pathological disorders may influence attentional allocation and conditioning rates, therefore certain questionnaires are employed to ensure participants are matched in experimental groups. This is important for studies whereby participants will undergo different experimental manipulations dependent upon which experimental group they are assigned to. Questionnaires used are shown in the appendix (appendix 2 to 7).

A monetary reward will be used as the appetitive reward, as money has been shown to be a useful substitute reinforcer in the absence of drugs, alcohol, cigarettes, etc, in

circumstances where it may not be appropriate to use these outcomes (Hogarth, Dickinson, et al., 2008). Money elicits many of the same responses as the other aforementioned reinforcers, and has the benefit of not being associated with some of the aversive properties linked with these other outcomes. In addition, during conditioning deprivation from nicotine can endow cigarettes with conditioned aversive properties (Hutchison, Niaura, & Swift, 1999), which may render them useless for measuring reward driven attention. Humans are also motivated to work for monetary rewards (Comer, Collins, & Fischman, 1997) and despite being traditionally viewed as a secondary reinforcer its ability to drive motivated behaviours is well documented as well as being able to mirror activation of brain areas associated with primary rewards (Elliott, Newman, Longe, & Deakin, 2003).

A blast of white noise is the chosen aversive reinforcer, as previous studies have demonstrated the ability of a white noise to give conditioned stimuli associated with the same noise the ability to generate CRs (Hogarth, Dickinson, Janowski, et al., 2008; Knight, Nguyen, & Bandettini, 2003). In addition, a blast of white noise has been shown to potentiate a motivated avoidance response in animals (Hughes & Bardo, 1981) and also in humans (Loeber & Duka, 2009).

Transfer will be conducted under nominal extinction, such that the outcome contingencies established during Pavlovian conditioning will no longer be in force and all stimuli will represent equal chance of the outcome. The outcome will, however, not be removed completely and will be scheduled to occur on a limited percentage of trials (equal for all stimuli) to encourage participants to continue to respond and prevent the instrumental responding from extinguishing entirely.

Negative mood

Decrease in reward sensitivity and depressive mood

Anhedonia is an important symptom of depression and is associated with decreased sensitivity to pleasurable events, and therefore reward, as described earlier. In example, one study (Rogers, et al., 2003) utilised acute tryptophan depletion to bring about negative mood and demonstrated the ability of state mood to manipulate processing of reward, but not punishment, cues. Volunteers who had undergone acute tryptophan depletion were seemingly less able to discriminate between expected gains in a gambling decision making task. The task involved several experimental gambles in which the participants had to select one of the two presented gambles in each trial. The participants were presented with both losses only, and gains only, trials, as well as experimental trials. One example of an experimental trial offered was a choice between a 25% chance of winning 80 points with a 75% chance of losing 20 points, versus a 50% chance of winning or losing 10 points. Despite an impact of mood on reward discrimination being shown, the results in this study did not demonstrate a link between negative mood and altered discrimination for expected losses. Furthermore, additional studies discussed below actually demonstrate a link between negative mood and increased punishment prediction (e.g. Blair et al., 2008). In contrast to the study discussed above (Rogers, et al., 2003), it has also been demonstrated that when faced with a choice between a likely smaller win and a less likely larger win participants experienced with successful tryptophan depletion do not significantly differ in their selection from those under control conditions (Anderson, Richell, & Bradshaw, 2003). This finding is unexpected because reduced serotonin is often linked to increased impulsivity so it would be anticipated that those under acute tryptophan depletion would make the riskier choice, and it is also interesting with regard to the impact of negative

mood (as brought about by reduced serotonin) on impulsive behaviours and instrumental responding.

Subjects under negative mood could therefore respond in differential ways during the crucial transfer stage of PIT in an appetitive paradigm. Firstly, they could be insensitive to the effects of reward value in the expression of conditioning and thus differential probabilities about reward become irrelevant and a reduced instrumental responding for that same reward may be seen generally as if the outcome had been devalued. Alternatively, a second hypothesis supports the possibility that those under negative mood may show an attenuated sensitivity to reward, and reduce instrumental responding for reward but maintain a differential level of responding in the presence of differentially predictive cues. Finally, it is also possible that subjects who were experiencing a negative mood state may not pay attention to the stimuli and such altered attention may interact with expression of the PIT effect. It is not clear that induction of negative mood will model anhedonia, however, it is generally accepted that by adopting certain procedures (i.e. manipulating serotonin and/or mood induction procedures), the effects of mood on several behaviours can be seen (e.g. Cools et al., 2005). It is also less clear how those under induced negative mood will respond in an aversive PIT model.

The main effects of mood manipulations

There is an abundance of studies investigating the effects of mood. One of the main methods used to induce state mood is serotonin manipulation, often coupled with mood induction, and these state mood manipulations have been shown to affect decision-making (Rogers, et al., 2003), motivation (Cools, et al., 2005), impulsivity (Clark et al., 2005), instrumental learning and information processing (Finger et al., 2007; Merens,

Willem Van der Does, & Spinhoven, 2007) as well as emotional and behavioural processing (Cools, Roberts, & Robbins, 2008). A selection of these studies will be discussed below and are summarised in Table 1 (see appendix 8). It is interesting to note that serotonin manipulation has been less extensively studied in currently depressed patients compared to healthy individuals but completed studies suggest results are not comparable between the two populations and different effects of serotonin reduction are observed (Harmer, 2008; Merens, et al., 2007). Furthermore, the use of lactose capsules as a placebo in some acute tryptophan depletion studies (Blair, et al., 2008; Finger, et al., 2007) coupled with consumption of low-tryptophan meals induced a lower tryptophan state in the controls than would normally be expected.

Despite wide acceptance of serotonin's role in many behavioural and emotional processes the exact contribution to these processes is not entirely understood (Cools, Robinson, & Sahakian, 2008; Harmer, 2008; Merens, et al., 2007). Manipulating serotonin has provided a method for studying its wide effects, and, more broadly, the effects of negative mood (e.g. Cardinal, Winstanley, Robbins, & Everitt, 2004). The production of serotonin in the brain is dependent on the precursor amino acid tryptophan, from plasma (Cools, Roberts, et al., 2008; Harmer, 2008). By providing participants with a mixture containing an amino acid load deficient in tryptophan this effectively results in a decrease in serotonin production (Harmer, 2008; Robinson, Cools, Crockett, & Sahakian, 2009). Another aspect to be taken into account is that similar reductions in serotonin have been observed in certain genetic polymorphisms as discussed below.

The impact of genetics and trait predispositions

Although not the purpose of this thesis, it is worth to note that certain individuals carry a polymorphism on the promoter region of the gene that encodes for the serotonin

transporter. It is possible for this to be mimicked in experimental animals both naturally, and brought about artificially though transgenic processes. This polymorphism controls the efficacy of the transporter and the promoter region affected was thought to contain both long and short allelic variants, but is now increasingly thought to be more complex; the short allele is associated with reduced expression while the long with increased, and interestingly the short allele has been linked to reduced serotonin function possibly brought about by developmental adaptations to the polymorphism (Cools, Roberts, et al., 2008). Studies involving investigation into volunteers with one or two copies of the short allele have demonstrated a link to anxiety and depression (Cools, Roberts, et al., 2008). As trait predispositions can impact upon the effects of mood certain questionnaires will be utilised, see appendix, to match participants in experimental groups for impulsivity and Beck Depression Inventory (BDI). It is important to match participants for BDI scores as the aim of this thesis is to investigate the effect of state mood, as opposed to trait mood.

The effect of mood manipulation on motivation

One study (Cools, et al., 2005) demonstrated the ability of tryptophan depletion to affect motivation on goal directed behaviour using a cued-reinforcement reaction-time task. In the task participants were shown three pictures and asked to select the "odd one out" and reinforcement of the outcome given on 10%, 50% and 90% of trials (dependent on the colour of the stimulus window). Those participants who had undergone tryptophan depletion showed reduced response speeds, related to increased reinforcement, but increased accuracy that correlated highly with their innate impulsivity as determined using the Barratt Impulsivity Scale. However, in the same study, tryptophan depletion did not alter response inhibition or mood as ascertained using the stop-signal task and visual analogue scales respectively. This demonstrates the ability of serotonin depletion

to impair response to incentive motivational cues that signal certainty of reinforcement primarily in participants who had greater innate impulsivity. Thus serotonin depletion may have a complex effect on behaviour.

Further to this, another study (Wogar, Bradshaw, & Szabadi, 1991) utilised naïve female Wistar rats that were trained in a standard operant conditioning chamber to lever press for sucrose solution as a positive reinforcer. The rats received specific lesions either as controls or targeted to damage the central serotonergic pathways, and were food deprived prior to experimental testing. The results gave evidence to the possibility of the involvement of serotonin pathways in controlling the value of reinforcers, such that damage to the serotonergic system increased the value of the reinforcer but did not impact upon overall responding. It is difficult to extrapolate these data directly to humans and as the data are in some conflict with other studies performed in humans (Robinson, et al., 2009; Robinson & Sahakian, 2009b) it enhances the complexity of the impact of serotonin and the serotonergic system on behaviour and, more specifically, motivation.

The effect of mood manipulation on positive bias

Similar to the aforementioned study, an additional study also utilised the cued-reinforcement reaction-time task (Robinson, et al., 2009), however this study implemented a mood induction procedure after successful acute tryptophan depletion. The mood induction procedure involved provocative sentences being displayed on a carefully selected coloured background whilst mood-inducing music was played through headphones. A further task was also completed, in the same study, monitoring the recall of self-referent words. In both tasks a positive cognitive bias was seen in subjects who had undergone positive mood induction that could be reversed using acute tryptophan depletion. However, under negative mood induction, tryptophan depletion

was able to induce a positive cognitive bias. These results indicate that mood plays a crucial role in modifying the effects of serotonin on emotion-related processes. Another study was able to further differentiate between serotonin and state self-reported mood (Robinson & Sahakian, 2009b). The study utilised acute tryptophan depletion and the mood induction procedure described above, followed by two tasks: A "hot" cognitive task involving processing of affective stimuli, affective go/no go, performance on which was affected by tryptophan depletion but not mood and a "cold" task, one touch Tower of London, that was affected by mood but not serotonin.

The effect of mood manipulation on behaviour

A study conducted in both Sprague Dawley and Brown Norway rats, (Jans, Korte-Bouws, Korte, & Blokland, 2008) monitored the effect of acute tryptophan depletion on behaviour (anxiety related behaviour tests [open-field test, home cage emergence test and social interaction test], depression related behaviour test [forced swim test] and cognition test [object recognition test]) and at the neurochemical level, showed strain differences in the effect of acute tryptophan depletion. The Sprague Dawley rats showed increased anxiety and depression related behaviour as well as reduced plasma serotonin, whereas the behavioural effects were not seen in the Brown Norway rats but they did show reduced plasma serotonin as well as reduced hippocampal serotonin, which was not observed in the other strain. This suggests that, like humans, certain innate traits observed across strains are required for the effects of serotonin depletion to be seen.

The effect of mood manipulation on inhibition and impulsivity

Whilst serotonin is thought to be involved in impulsive decision making (Clark, et al., 2005; Cools, Roberts, et al., 2008; Cools, Robinson, et al., 2008), its role in inhibition was investigated in a study (Clark, et al., 2005) reported in 2005, hence investigating the link between negative mood and impulsive decision making. In addition to utilising

acute tryptophan depletion, the study investigated the effect of the serotonin transporter polymorphism and how subjects with this polymorphism (subjects with both one and two copies of the short allele) respond differently to tryptophan depletion. It was proposed that this polymorphism leads to inter-subject variation may account for the variation in reported results in previous acute tryptophan depletion studies. The study consisted of 41 subjects comprising of the three possible serotonin transporter genotypes (15 long-long, 19 short-short and 7 short-long participants) but no effect of treatment (placebo/acute tryptophan depletion) or genotype was observed with regard to inhibitory performance on the stop-signal task. Additionally the effect of acute tryptophan depletion on the task was not modulated by trait impulsivity or gender, but with placebo treatment the results measured by the Barratt Impulsivity Scale correlated with the stop signal response task times. The results of this study therefore do not show a direct correlation between increased impulsive behaviour and reduced serotonin transmission, but as only one task was utilised the theory cannot be fully refuted, and as no effect of genotype was observed this further dissociates impulsivity and serotonin neurotransmission. It is important to note that the task utilised does not incorporate feedback, reward or punishment and these may be important features in the role of serotonin and mood in impulsivity as described in other studies.

In contrast, (Mobini, Chiang, Ho, Bradshaw, & Szabadi, 2000) demonstrated that when challenged with the choice between a smaller immediate reward (selection of lever A for one food pellet) and a larger delayed reward (selection of lever B for delayed delivery of two food pellets), rats who had undergone destruction of their serotonergic pathways showed an increased tendency toward responding on lever A linking serotonergic system disruptions and increased impulsivity. Mobini et al (2000) also presented an additional experiment in which rats selected between lever A (as described

above) and lever B, which now represented the delivery of two pellets but with a lower probability than reward from lever A. A difference between control and experimental conditions in the second experiment was not observed, indicating no impact of serotonin depletion on sensitivity to probabilistic reinforcement in rats.

The interaction of mood on reward and punishment sensitivity

There are a variety of studies utilising acute tryptophan depletion to manipulate serotonin and study the effects of mood, however there are other methods by which it is possible to investigate these effects (Cools, Roberts, et al., 2008; Merens, et al., 2007). These include the use of a selective neurotoxin for serotonin in animal studies, the use of selective serotonin reuptake inhibitors and by studying the genetic polymorphisms that affect the serotonergic system. It is generally accepted that mood, via serotonin, mediates behavioural inhibition (Cools, Roberts, et al., 2008; Merens, et al., 2007) and is involved in aversive learning and punishment such that a reduction in serotonin enhances aversive processing, and neural activity in the amygdala is potentiated by this reduction (Cools, Roberts, et al., 2008). It is possible that this process might be in opposition to the appetitive dopaminergic activity involved in motivational processes. Further to this, Pavlovian conditioned inhibition, as well as other forms of inhibition, may be modulated by serotonin (Cools, Roberts, et al., 2008) and therefore be linked to mood. These results may not be detectable by the mild reduction in serotonin caused by acute tryptophan depletion.

One study (Finger, et al., 2007) investigated the effects of serotonin transporter genotype and tryptophan depletion on reward and punishment using response reversal and passive avoidance tasks respectively. Participants consumed either placebo or capsules inducing acute tryptophan depletion and also underwent genotyping for the serotonin transporter. The passive avoidance task involved 12 possible stimuli of which

six led to reward (increased points) and six lead to loss of points (punishment). The participants learnt by trial and error to respond (press the mouse button) to positive stimuli and not respond to punished stimuli (feedback was provided after each selection). The successful avoidance of punished stimuli did not correlate to treatment but improved throughout blocks and there was no correlation between treatment and genotype; however on analysing genotype alone long/long carriers learnt slower and for longer compared to the short allele carriers, whose learning was quicker and plateaued after the fifth block. There was an effect of treatment on the total number of non-responses to rewarded stimuli, such that those participants who had undergone acute tryptophan depletion missed more positive stimuli than those who had taken placebo, but no effect of genotype was seen here. The probabilistic response reversal task involved selection of a stimulus from a displayed pair (pairs were displayed together throughout the task) and after selection participants were given feedback as to whether they had lost or gained one hundred points dependent on their selection. The rewarded stimuli remained constant in four out the six pairs of stimuli, however in the other two pairs the contingency reversed after forty of the eighty trials had been completed; additionally in these "reversing pairs" the contingency was not 100% such that on 20% of trials the positive stimulus was punished and the negative stimulus was rewarded. No effect of treatment alone was observed, however, long-long allele carriers who had undergone tryptophan depletion committed more errors than both long-long carriers on placebo and short allele carriers. In addition long-long carriers were also less likely to return to selection of the positive stimuli in the reversing pairs once it had been punished. The results suggest that although tryptophan depletion has the ability to affect aspects of tasks utilised in this study, the interaction with genotype proved more significant and this may account for inconclusive results found in previous studies. One

possible explanation for the increased effects of tryptophan depletion in long-long allele carriers could be increased serotonin reuptake by these genotypes and thus enhancing the effect of acute tryptophan depletion (Finger, et al., 2007).

Another study (Blair, et al., 2008) found serotonin to be particularly involved in sensitivity to punishment rather than to reward and that sensitivity to tryptophan depletion was, in some circumstances, modulated by serotonin transporter genotype. In the task selected for this study (differential reward/punishment task), pictures of objects were randomly assigned a points value (100, 300, 500, 700, 900, -100, -300, -500, -700 or -900) and pairs were presented to the participant. Participants were asked to select one of the two objects presented. Feedback as to the value of the object they had selected was immediately presented, but they were not told the value of the other object or if the object they had chosen was the more advantageous. There were trials on which both objects were associated with points losses ("punpun") or points gains ("rewrew") and also combination trials ("rewpun"). Additionally, the trials were further sub-divided into close, medium and far in which the points values of the two objects were similar, fairly different and very dissimilar respectively. Genotype was shown to have no effect on levels of tryptophan after intentional depletion or placebo. Overall, participants were more accurate on the far trials and most accurate on "rewpun" followed by "rewrew" and made most errors on "punpun" trials. Acute tryptophan depletion did not affect the errors produced by the short allele carriers but increased the errors produced by long-long carriers when compared to placebo. The results of the task demonstrate that serotonin is involved in punishment processing, rather than that for reward and that serotonin transporter genotype is involved in sensitivity to acute tryptophan depletion and the ability to process punishment and make related decisions. This provides an

important link between mood and reward/punishment outcome processing, which I will aim to build upon.

Summary of the effects of mood manipulations

Interestingly, in addition to the important distinctions discussed above it is important to note that mood induction procedures are seemingly required for the effects of induced state mood to be examined and that acute tryptophan depletion alone may not be sufficient for participants to express altered state mood (Robinson, et al., 2009). Due to this distinction, a mood induction procedure (MIP) will be utilised to induce a state mood that can then be examined using the general PIT model. A recent renewed interest in the link between emotion and cognition has lead to the development of many MIPs (Gilet, 2008). Of the currently adopted techniques, the earliest involves reading aloud self-referent sentences and is known as the Velten Mood Induction Procedure (Velten, 1968). Other methods involve the use of music, film clips (Gouaux, 1971) and combinations of different methods are also utilized (Robinson, et al., 2009). It has been proposed that one of the most effective combination techniques involves imagery and music (Gilet, 2008), and a similar combination will therefore be used to induce state mood in this thesis. Another reason for this selection is that some methods, in particular those utilizing self-referent techniques such as the Velten procedure, have been criticized for bringing about induced mood as a result of demand effects, although it is difficult to provide conclusive evidence regarding this impact (Gilet, 2008).

It is possible that those in a negative mood state may be more analytical of their situation and choices available to them such that they demonstrate increased consideration of decisions leading to reduced speed but better accuracy in tasks undertaken; contradictory findings may be accounted for by the lack of either genotyping of participants, mood induction, analysis of innate impulsivity and/or

successful placebo control groups. It is also possible that studies demonstrating a link between serotonin and response to negative consequences of reward seeking, as well as impulsivity, may be explained by innate impulsivity. The argument as to whether negative mood affects punishment and/or reward (gains) information processing still appears undecided and clarification is imperative if the affects of anhedonia are to be successfully studied. Finally, it is therefore possible that negative mood in isolation may interact more with changes in reward value than negative consequences of reward seeking.

In summary, the effects of mood (mediated by serotonin) are vast, but the processes that mediate these effects do not appear to be fully understood. In order to gain a fuller picture of the true effects of acute tryptophan depletion, innate impulsivity should be considered and genotyping of participants may be required. Mood induction procedures may therefore offer a simpler tool to modify mood as it represents a more naturalistic method of inducing a state mood, and will be utilised in the studies described in this thesis.

General Materials for the Mood Induction Procedure

A musical and visual Mood Induction Procedure (MIP) will be utilised to induce positive and negative state mood (as well as a neutral version as a control condition), adapted from a previously described method (Robinson, et al., 2009).

Participants will be presented with 44 images (Lang, et al., 1993; Smith, et al., 2005) whilst music is played through Sennheiser PX200 headphones and instructed to get as deeply as possible into any mood evoked. Firstly, a blank screen will be presented and participants instructed to press the space bar to view the first picture, and to look at it

for as long as it is displayed. The picture will be displayed in the centre of the screen for 12 seconds, immediately followed by a blank screen. When the blank screen is displayed again participants will press the space bar to view the next picture. For images please see appendix 9, appendix 10, appendix 11 and appendix 12. The music played will be *Adagio in G Minor* by Thomas Albinoni for the negative version of the MIP, *Serenade No.13 KV 525 G Major: I. Serenade. Allegro* by Wolfgang Amadeus Mozart for the Positive version of the MIP and for the neutral MIP *The Planets, Po. 32: VII. Neptune, the Mystic* by Gustav Holst.

Prior to and after the MIP, a set of Visual Analogue Scales (VAS) will be administered (see appendix 7), to determine self reported mood. Comparison between initial- and post- MIP self reported mood, using VAS, will be used to determine the mood effects of the MIP and therefore assess the effectiveness of the procedure.

Paper 1 Summary

The MIP described above will be introduced in paper 1, which presents a series of two experiments designed to build upon the previous research suggesting that negative mood differentially interacts with aversive and appetitive outcomes, and to address some of the uncertainties surrounding the effects of negative mood on motivation. Specifically, we set out to determine the effect of induced state negative mood on PIT by stimuli associated with reward (study 1) to further understand the role of anhedonia on motivated behaviour, and by stimuli associated with aversive outcomes (study 2) to increase our knowledge on the impact of mood on punishment prediction. In addition, since paper 2 will utilise a reward devaluation technique in the same PIT design as for study 1, it will allow us to investigate more deeply the mechanisms by which negative

mood affects goal-directed behaviour. The set of experiments in paper 1 allows us to draw direct comparisons between not only state neutral and negative mood, but also between our ability to respond to aversive and rewarded stimuli. The motivation to avoid punishment/gain reward when under negative mood can also be observed.

This will then be built upon in paper 3, which aims to investigate the effect of positive mood on PIT.

Value of reward as a mediator of goal directed behaviours

Expectancy and attentional bias

Both the Mackintosh (Mackintosh, 1975) and Pearce Hall (Pearce & Hall, 1980) theories of learning outline the requirement of attention as important in acquiring knowledge of a stimulus/reward association albeit differing in their theory of attentional bias once some knowledge of the association has been formed (discussed in detail later). The resulting expectancy of a certain outcome is important for learned behaviour in both theories, and it is thought that reward seeking behaviour is controlled by the expectancy of a reward, which may be cognitively mediated, and also linked to CRs and physiological responses (e.g. skin conductance response) (Hogarth, et al., 2005; Hogarth, Dickinson, Hutton, Bamborough, et al., 2006; Hogarth, Dickinson, Hutton, Elbers, et al., 2006; Hogarth, Dickinson, Janowski, et al., 2008). Expectancy is formed on the expected probability that an action will produce the reward but also on the expected biological value of the reward (Hogarth, Dickinson, Hutton, Elbers, et al., 2006; Wertz & Sayette, 2001). In example, in one study smoking cues only elicited craving (an important response associated with addiction) for the same reward if participants felt they would be able to smoke after the experimental session in which

they experienced the cue; in contrast the same study demonstrated that those who thought they could not smoke after the session did not show enhanced craving (Droungas, Ehrman, Childress, & O'Brien, 1995). Taken together these studies demonstrate that conscious expectancy of the US is related to cue-elicited behaviour, and therefore that awareness is required for successful conditioning in many paradigms. However, under some conditions evaluative conditioning may occur in which explicit expectancy, or outcome awareness, is not required and CRs occur seemingly independently. This form of evaluative learning is often observed when using food or drink as the rewarding stimuli. In example, when a novel flavoured drink (CS) was paired with caffeine (US) liking for the drink was enhanced compared to when the drink was not paired with caffeine (Yeomans, Spetch, & Rogers, 1998). None of the participants who had consumed the caffeine paired drink reported awareness of the caffeine, thus this form of learning refers to changes in the liking of a stimulus as a result of pairing with another stimulus and outside of contingency knowledge.

In parallel to the role of expectancy in cue-elicited behaviour for reward, conscious expectancy of a US has been shown to influence conditioned aversive responding in the presence of CS. In example, high expectancy ratings were indicative of high skin conductance responses in the presence of CS when using images of snakes and spiders (Ohman, Eriksson, Fredriksson, Hugdahl, & Olofsson, 1974).

Additionally, emotional learning has been hypothesised to occur prior to, and independently from, conscious stimulus contingency awareness in the somatic marker hypothesis (Damasio, 1996), and has been further demonstrated when a tone paired with an aversive white noise elicited a skin conductance response academic of expectancy of the US (Knight, et al., 2003). Whilst the somatic marker hypothesis pertains to

reasoning and decision-making processes, these are also important factors in addiction processes and can be related to conditioning and thus should not be ignored.

Attention to cues is important in the classical conditioning process and is concurrent with addiction to alcohol (Townshend & Duka, 2001) as well as drugs of abuse (Lubman, Peters, Mogg, Bradley, & Deakin, 2000) and food. Increased attention is also linked to increased chance of relapse and craving (Cousijn et al., 2013; Garland, Froeliger, Passik, & Howard, 2013; Werthmann, Field, Roefs, Nederkoorn, & Jansen, 2014). The core tenet of attention to drug cues is that it is enhanced by the incentive or pleasurable properties of the drug (Robinson & Berridge, 1993). It is widely accepted that attention is automatically allocated to such cues, whilst one theory also proposes attention is maintained facilitating craving and relapse (Franken, 2003), and another describes the process whereby cues are pre-evaluated and if deemed suitable (i.e. a drug cue) selective attention is apportioned to them (Ryan, 2002). Therefore, according to the latter, if a cue is not deemed relevant a reduction of attentional resources would be apportioned to the stimulus and thus diminish the chance of craving. When the stimuli do attract attention this may induce drug seeking by inducing expectancy of the rewarding properties of the drug (Robinson & Berridge, 1993) or by activating a more general motivational pathway (Tiffany, 1990). Indeed, understanding how attention is apportioned to predictive stimuli is important to teasing apart the attentional mechanisms involved in undesirable behaviours and pathological disorders. Furthermore, it is hypothesised that a central motivational state is activated in response to both rewarded and punished stimuli (Bindra, 1969) underpinned by a union of motivation and emotion; by studying the process of Pavlovian learning using participants who have undergone mood manipulations the effect of state mood on these

mechanisms can be investigated. As mood is strongly linked with motivation this interaction will be an interesting process to observe.

Various studies in which attentional bias for both certain and uncertain stimuli has been monitored show that contingency knowledge is required for the stimuli/reward association to be learned (Hogarth, et al., 2005). Indeed, expectancy should also be coupled with an appetitive emotional response that is indicative of the positive biological value of the reward (Hogarth & Duka, 2006a). However, it has also been demonstrated that a positive attentional bias for the stimulus is not essential for establishment of an instrumental response (Hogarth, Dickinson, Janowski, et al., 2008) and thus an impulsive aspect may be considered. The afore mentioned studies demonstrate a level of uncertainty in the way in which attentional bias is affected by increasing knowledge of the outcome of the predictor (Hogarth, Dickinson, et al., 2008; Hogarth, Dickinson, Janowski, et al., 2008). However, the role of expectancy appears more defined and it seems risk and ambiguity may also play a role in learning (Schultz et al., 2008b). It seems possible that expectancy and attentional bias may not be directly linked (Hogarth, Dickinson, et al., 2008). It does, however, seem clear that it is the expected reward, rather than a mental representation of the reward that drives the behaviour. Under certain conditions, when dependence has progressed sufficiently, altering the value of the reward has limited effect on the behaviour to obtain the reward and despite negative outcomes of this behaviour compulsive seeking occurs (Economidou, et al., 2009; Everitt & Robbins, 2005).

In paper 2 the impact of a change in the expected value of the reward on reward seeking will be examined using a reward devaluation procedure, to advance our knowledge of the processes involved in goal-directed behaviours. The attention to the stimuli will be assessed using observing times, which will allow an insight into separating salience and

attention from prediction error, and thus investigate the link between learning and attention in a human PIT model.

Decrease in reward sensitivity and addiction

Addictive behaviours of a compulsive nature are suggested to be insensitive to the negative consequences of rewarded behaviour (Economidou, et al., 2009; Everitt & Robbins, 2005). Individuals with certain addictive behaviours show an inability to modulate established rewarded behaviour following a reduction in the value of the outcome of that behaviour, instituted by either non-reward (extinction) or punishment (Belin, Mar, Dalley, Robbins, & Everitt, 2008; Economidou, et al., 2009; Pelloux, et al., 2007; Vanderschuren & Everitt, 2004).

Reward devaluation

Findings from recent human studies on PIT trying to address the question of the role of the current reinforcer value in the instrumental reinforcer-seeking response are contradictory. Whereas Allman et al, 2010 (Allman, Deleon, Cataldo, Holland, & Johnson, 2010) showed that outcome specific transfer can be modulated by the current value of the reinforcer, Hogarth and Chase, 2011 and Hogarth 2012 (Hogarth, 2012; Hogarth & Chase, 2011) showed no effect of devaluation in a reinforcer specific transfer paradigm. The studies differed in the way they ran the PIT procedure: Allman et al induced devaluation by instruction (Allman, et al., 2010). Participants learned in the Pavlovian phase that companies (presented by their logos) were using Hong Kong or USA dollars as their currencies. Before the transfer phase participants were informed that one of the currencies used was worthless (devalued). This information modulated

the choice response when the company logos were presented in the transfer phase: cues that predicted the devalued currency lost their capacity to strengthen the choice response for this outcome. Hogarth (2012) and Hogarth and Chase (2011) used as conditioned stimuli pictorial representations of the reinforcers (cigarette or chocolate), to which participants had been exposed several times pre experimentally. Devaluation of cigarette reinforcer (nicotine treatment) and chocolate reinforcer (offered chocolate to eat) did not change the reinforcer choice responses.

Suppression of drug-seeking by punishment or conditioned suppression has also been reported in animal studies (Stephens et al., 2005; Vanderschuren & Everitt, 2004), however it would appear that an extended drug-taking history appears to render the ability of aversive stimuli or outcomes unable to reverse instrumental seeking responses. This was assessed in various studies, including (Vanderschuren & Everitt, 2004) where a footshock-paired conditioned stimulus was unable to significantly reduce cocaine seeking after prolonged exposure in rats; this was further investigated in the same study to assess whether the incentive value of cocaine was the causal factor in this inability to reduce seeking but it was found that there was no difference in the prolonged and limited exposure groups in regard to the value of cocaine.

An additional study conducted in rats (Pelloux, et al., 2007) demonstrated that both food (sucrose) and drug (cocaine) seeking were reduced by punishment following moderate lengths of exposure, but that following extended exposure of cocaine there was an observed inability of the punishment schedule to reduce the seeking response. It was highlighted in this study that a sub-population of rats were particularly susceptible to persistent drug-taking despite punishment. This was assessed in a further study (Economidou, et al., 2009) in which rats were initially assessed for innate impulsivity and then relapse to cocaine through punishment was tested. The effect seen was that

highly impulsive animals demonstrated a greater likelihood to relapse than their low-impulsive counterparts. These studies outline that both a pre-existing innate impulsivity as well as length of exposure are factors involved in leading to impulsive drug seeking despite negative consequences. It appears that this has yet to be extensively studied in humans (Belin, et al., 2008), however, in animals it has been shown that certain innate traits associated with low concentrations of dopamine $D_{2/3}$ receptors may make for a pre-disposition for vulnerability to drug use and addiction (Dalley, et al., 2009). Moreover, changes in the same receptors in the nucleus accumbens may be associated with impulsivity while the dorsal striatum was also hypothesised to be implicated in development of such behaviour (Dalley, et al., 2009).

Regions of the brain associated with addiction

Although not the scope of this thesis, PIT is anatomically dissociable from certain similar processes, and therefore associated anatomy will now be discussed. Addictive behaviour has often been linked to the nucleus accumbens and dopamine inputs from the ventral tegmental area into the nucleus accumbens play an important role in addictive processes (Dalley, et al., 2009). However, frontal brain areas that include the orbital prefrontal cortex and other limbic regions are susceptible to addictive drugs and may play a role in compulsion and impulsivity (Dalley, et al., 2009; Everitt, et al., 2007). Lesions to the orbital prefrontal cortex impair drug-seeking behaviour both pre- and post- Pavlovian association formation and it is possible that impaired orbital prefrontal cortex function may be linked to impulsivity and impaired decision making (Everitt, et al., 2007; Everitt & Robbins, 2005). Lesions to the basolateral amygdala and the nucleus accumbens core render rats unable to perform a drug-seeking task (Cardinal, et al., 2004; Everitt, et al., 2007).

It has been shown experimentally through specific lesions that the basolateral amygdala is involved in conditioned reinforcement, second-order conditioning and second-order instrumental responses during appetitive conditioning as these processes are sensitive to lesions in this area. However lesions in the central nucleus affect PIT and therefore the association of the stimulus to the response in both aversive and appetitive conditioning (Everitt, et al., 2003). During aversive conditioning behaviour is insensitive to lesions in the central nucleus but affected by lesions to the basolateral amygdala; both types of lesions affect the startle and freezing response (Everitt, et al., 2003).

As discussed above several regions within the brain are involved in the development of learning and addiction, leading to habit and compulsion. More specifically these include the amygdala, the nucleus accumbens core, the orbital prefrontal cortex and also the hippocampus; it has been shown that inactivation of the dorsal hippocampus can prevent reinstatement of an extinguished response to certain drugs. Additionally, mesolimbic dopamine modulates interactions of projections into the nucleus accumbens from the amygdala, prefrontal cortex and the hippocampus (Everitt, et al., 2008; Everitt & Robbins, 2005).

Drug taking is associated with dopaminergic innervation of the ventral striatum and the nucleus accumbens shell and is shifted to drug-seeking with the accompaniment of the integrity of the nucleus accumbens core and its afferents from the basolateral amygdala. The development of impulsive drug seeking may be marked by innate impulsivity perhaps, but also correlates with low numbers of available dopamine receptors in the ventral striatum and the nucleus accumbens. Established impulsive behaviour is characterised by reduced numbers of dopamine $D_{2/3}$ receptors in the ventral striatum and dysfunction of the orbito-prefrontal cortex, perhaps innate but potentially caused by even limited drug exposure (Everitt, et al., 2008).

The impact of a change in the actual value of the reward on reward seeking will be examined in paper 2 as previously mentioned.

Paper 2 Summary

Paper 2 presents a study demonstrating a devaluation technique used in combination with appetitive PIT, designed to further our understanding of the conditions under which current reinforcer value can modify responding to a conditioned stimulus associated with that reinforcer. The PIT procedure utilised appetitive probability based conditioning and although in such a procedure stimuli acquire different strength associations with the reinforcer and they are therefore able to activate response accordingly by signalling the probability by which the reinforcer can occur, they do not have any capacity in signalling perceptual characteristics of reinforcer that are remote from its value (reinforcer retains for each of the stimuli the same perceptual characteristics). Additional measures were taken of attention and emotional reactivity to clarify the involvement of attentional orientation to the stimuli and emotional conditioning response in contributing to the transfer effects.

As negative mood is associated with an attenuated sensitivity to pleasurable events it is possible that the results observed in paper 1 when under negative mood may mimic the effects of reward devaluation in relation to response to altered reward (or perceived reward) value.

The impact of positive mood on instrumental behaviours

Affective disorders have traditionally been studied using either healthy volunteers who have undergone mood state manipulation/induction, or patient populations. Whilst it is generally accepted that inducing a negative mood state mimics mild depression (Clark, 1985) the effects of positive mood, and the effectiveness of positive mood induction, is not as well established. Studies into the effects of induced state positive mood is particularly interesting with regard to the PIT model, as one of the symptoms of mania is abnormal decision making (Clark, Iversen, & Goodwin, 2001). Certain lesions in the ventral pre-frontal cortex render patients with mania like symptoms of impulsive decision-making, often despite negative (or punishment) outcomes (Drevets et al., 1997). This region is also believed to be involved in mood disorders (Goodwin et al., 1997), as well as mood more generally (Baker, Frith, & Dolan, 1997).

If the ventral pre-frontal cortex and impulsive decision-making are implicated in mania, and extreme positive mood, it would be reasonable to assume that positive (as characterised by increased impulsive decisions) and negative [as characterised by reduced sensitivity to pleasurable events (Henriques, Glowacki, & Davidson, 1994) as discussed earlier] mood would have opposing effects on certain gambling tasks [i.e. such as the tasks presented in (Bechara, Damasio, Damasio, & Anderson, 1994; Steingroever, Wetzels, Horstmann, Neumann, & Wagenmakers, 2013)]. However, this was not found to be the case when tested in a study reported in 2001 (Clark, et al., 2001) that utilised "the Gambling Game" in which participants had to select from four decks of cards where two of the decks were associated with high wins but high losses, and the other two lower wins but also lower losses as no difference was observed between positive and negative state induced mood. This finding could perhaps indicate that induced positive and negative mood states influence decision-making in the same way,

but this was refuted by the authors, who instead suggested that trait positive mood was responsible for the impulsiveness seen in mania. This hypothesis was supported by a correlation between choices on the Gambling Game and questionnaire scores, however with an absence of a neutral mood condition it could not be confirmed. Indeed, the link between certain abnormal disorders and mood is well established (Hartley & Phelps, 2012) and makes for an interesting and important focus of study, especially with regard to future interventions. Further clarification of the impact of positive mood is required to develop our understanding of its affects on motivated actions and undesirable behaviours and this is investigated in paper 3.

Paper 3 Summary

In an attempt to increase our awareness of the effects of positive mood, paper 3 presents an experiment in which a mood induction procedure designed to induce positive (and neutral as a control condition) mood is employed prior to the transfer stage of the PIT design used throughout this thesis.

Specifically, we were keen to determine the effect of positive mood on motivation to perform an avoidance response to further our understanding on impulsiveness associated with positive mood, and the mechanisms that drive motivated behaviour in humans. The data will also provide a useful comparison to the previous experiments, presented in paper 1, which analyse the effect of negative mood in the same paradigm.

As mania is often associated with impulsive decision-making (Bentall, 1992; Clark, et al., 2001) the ability of positive mood to interact with acquiring S-O contingencies is also interesting and will be addressed in paper 4, along with the impact negative mood may have on these processes.

The effect of mood on learning S-O contingencies and attention to the CS

As discussed earlier negative mood impacts a wide variety of factors and is involved in abnormal behaviours, in particular drug addiction. Instrumental responding in the presence of CS, as in successful PIT, demonstrates a motivational aspect and this interaction with mood is investigated in papers 2 and 3. The exact mechanism by which this interaction occurs is a major focus of this thesis and is further discussed later in chapter 2.

Before an instrumental action can be associated with a stimulus and/or outcome, Pavlovian learning must have occurred. Classical conditioning is important in drug addiction processes, and the expectancy of the reward, as well as the biological expectancy coupled with appropriate attention, is also important to drive this process, as discussed earlier. Therefore, cognitive expectancy is often considered vital in Pavlovian reward learning and is well documented; for example, it has been demonstrated that only participants who become aware of outcome-contingencies (assessed using self reported expectancy ratings) elicit CRs in a CS-cigarette designed study (Hogarth, Dickinson, Hutton, Elbers, et al., 2006).

General emotional theories of attention aside, learning processes may also mediate attention and it can be conceptualised that attention, in turn, plays a key role in learning. Indeed, one model of learning describes that learning occurs by the number of times a stimulus and outcome are paired, such that learning is driven by increasing the salience of the stimulus thus attracting more attention (Mackintosh, 1975). Therefore, in isolation, this theory would assume that attention to a CS would be maintained even

after the outcomes are fully learnt and maintain their predictability. In contradiction, other ("prediction-error") models describe that once such predictability is reached attention to the stimuli will diminish (Pearce & Hall, 1980). According to this model of prediction-error the discrepancy between the occurrence of the outcome and expectancy attracts attention, such that the greater the error the more attention is apportioned in an attempt to facilitate learning. Therefore this model also assumes that attention will be modified throughout the learning process as outcome contingencies are learnt, and eventually attention will become automatic so that processing of the CS is just sufficient to maintain existing associations. However, should the CS cease to accurately and consistently predict the outcome then attention will increase and a switch from automatic to controlled processing of attention would occur. In summary, one theory predicts attention is biased towards uncertain predictors (Pearce & Hall, 1980), whilst the other hypothesises attention favours predictive salience (Mackintosh, 1975).

As discussed earlier dopamine neurones are responsive to reward (Fiorillo, Tobler, & Schultz, 2003; Schultz, et al., 2008b), but there is evidence to suggest they do not respond to aversive outcomes (Mirenowicz & Schultz, 1994, 1996; Robinson & Berridge, 2003). In contradiction to this, negative mood, and serotonin, are thought to be involved in learning regarding aversive outcomes (Cools, Robinson, et al., 2008; Dayan & Huys, 2008; Rogers, et al., 2003), and, are also linked to prediction error (Dunsmoor, Bandettini, & Knight, 2008).

Serotonin is also critical in mood, and negative mood has been associated with the concept of depressive realism (Robinson, et al., 2009; Robinson & Sahakian, 2009a, 2009b) and has also been implicated in changes in learning in both humans (Chase et al., 2010; Ruhe, Mason, & Schene, 2007) and animals (Wilkinson, Humby, Robbins, & Everitt, 1995). Chase and colleagues (Chase, et al., 2010) demonstrated that despite

acute tryptophan depletion generating no effect on their contingency learning task, a sub-group of participants with low BDI scores showed such an effect. Therefore, in paper 4 (and throughout) we match experimental groups for BDI score and manipulate mood in order to observe the effects of state mood.

General Materials for Eye-Tracking

Eye-tracking is a useful tool for measuring selective attention, as it is not confounded by the length of stimulus duration, as in other designs such as dot-probe tasks. Dot-probe measures attention during specific set time points, whereas eye-tracking follows movements throughout the whole stimulus duration and has been reported to provide more robust results (Isaacowitz, Wadlinger, Goren, & Wilson, 2006). In our design stimulus pairs are presented on each trial and therefore the amount of attention allocated to one stimulus over another is important to measure, and this measure is defined as selective attention for that stimulus (Posner, 2012).

The dwell time allocated to one stimulus over another presented concurrently is also an important measure of learning (Hogarth, Dickinson, et al., 2008), and conscious attention is also measured in our study by allowing the participant to view the stimulus pair for as long as they desired (Hogarth, Dickinson, & Duka 2009).

Paper 4 Summary

Following on from the investigations into the effects of mood on PIT in papers 1 and 3, paper 4 aims to build upon our knowledge in this area, whereby the effect of induced state negative and positive mood is investigated in a Pavlovian learning design. Paper 4 presents a combined study designed to further our understanding on the impact state

mood plays on learning and more specifically acquiring Pavlovian contingencies predictive of reward. The speed of learning outcome contingencies, and emotional responses to the cues are assessed, as well as attention to cues using eye-tracking. Attention is an important factor in learning (Hogarth, Dickinson, et al., 2008; Hogarth, et al., 2005; Hogarth, Dickinson, Janowski, et al., 2008) as well as enhanced attention being linked to pleasurable cues (Robinson & Berridge, 1993; Stewart, de Wit, & Eikelboom, 1984) which is interesting with regard to the link between mood and sensitivity to reward (as discussed above).

We used a Pavlovian conditioning design as described in papers 1, 2 and 3, but with an extended learning phase to enable all participants with the opportunity to successfully acquire Pavlovian contingencies. Slower learning was associated with induced negative mood and it is argued that this could be as a result of reduced motivation or reduced confidence linked to reduced impulsivity.

The aim is therefore to build upon the previous research suggesting that negative and positive mood differentially interact with learning processes, and to address some of the uncertainties surrounding the effects of state mood on sensitivity to reward and motivation. Specifically, we set out to determine the effect of induced state negative, and positive mood on Pavlovian learning when stimuli are associated with a rewarded outcome.

Chapter 2

General Discussion

Summary of findings

In chapter one, I reviewed the literature on the effects of manipulated mood on aspects of cognition and, also, described the mechanisms underlying reward seeking behaviour. I suggested that motivation to perform an instrumental response would be mediated by state mood. I proposed to investigate this hypothesis by adopting a classical conditioning procedure in which visual stimuli predicted a reward (or aversive) outcome with varying probabilities incorporated into a PIT design in combination with a mood induction procedure. I predicted that negative mood would be implicated in motivational processes, and participants induced with negative mood would be insensitive to the value of reward despite successful learning of the contingencies having occurred. Furthermore, in chapter one, I also described the phenomenon by which mood is implicated differently in punishment and reward prediction.

In paper 1, described in chapter 3, I set out to investigate the effects of induced state negative mood on mechanisms of motivation to gain reward and avoid adverse outcomes. Initially, participants underwent a Pavlovian learning procedure in which three visual compound stimuli (AX, BX and CX) predicted a monetary reward of 10p (study 1), or an aversive outcome of white noise (study 2), on 90%, 50% and 10% of presentations respectively. This was followed by a mood induction procedure (inducing state negative or neutral mood), a short phase of instrumental training and finally, transfer performed under nominal extinction. Each trial encompassed an expectancy question in which participants were required to express how likely they felt they were to receive the outcome on that particular trial, and the session was concluded with participants providing subjective ratings of emotional properties of the stimuli. Results of expectancy ratings did not differ between induced mood conditions in any stage of

the experiment, and were maintained into the transfer test by participants who were deemed aware of the contingencies by reporting an appropriate discrimination between the stimuli during Pavlovian learning. Attention to the stimuli was also not impacted by the mood induction procedure, but did demonstrate a shift between the learning and transfer stages of the experiment such that during Pavlovian learning observing times were greatest for the BX (uncertain 50% predictor) and reversed during the transfer test, supporting the prediction error hypothesis of attention. The same pattern for expectancy and attention was observed in both study 1 and study 2. The number of instrumental responses performed by participants in the neutral mood condition for each trial type (AX, BX or CX) increased linearly in accordance with the expectancies/probabilities learned (AX>BX>CX), in both studies, indicating the transfer effect is related to explicit prediction of the outcome. Interestingly, the transfer effect was altered in both studies by induced negative mood, such that the linear nature of the effect was abolished in study 1 (but not study 2), and a reduced number of responses observed compared to the neutral mood condition. The data therefore demonstrate a dissociation between attention (uncertainty) and behaviour (prediction) with regard to the effect of induced negative mood state on PIT. The differential impact of negative mood on the linear nature of the transfer effect in the two studies also highlights a difference between the impact induced negative mood may have on punishment and reward prediction, however the effect on PIT observed in study 1 (reward outcomes) may be confounded by mood reducing sensitivity to the expected gains and thus devaluing the reward.

In paper 2, described in chapter 4, I set out to investigate confounds from paper 1 and investigate if reward devaluation would impact on the PIT effect in the rewarded PIT design used in paper 1 (study 1). Participants again underwent a Pavlovian conditioning procedure in which three compound stimuli, AX, BX and CX, predicted a reward

outcome of 10p on 10%, 50% and 90% of presentations respectively, followed by instrumental training and a transfer test as for paper 1. Contrary to the previous study, a mood induction procedure was not adopted, but the value of the outcome was manipulated such that it was, either devalued, revalued or remained unchanged (as described in Chapter 4). Expectancy of the outcome was in accordance with the contingencies (AX>BX>CX) and maintained into the transfer test by aware participants across all experimental conditions. Additionally, there was no effect of mood manipulation on observing times, and as for paper 1 observing times were greatest for BX (over AX and CX) during training and lowest for BX during transfer, again supporting the prediction error hypothesis of attention. Interestingly, even participants deemed unaware of the contingencies because they did not successfully discriminate between the outcomes during Pavlovian learning showed an attentional bias to the stimuli in the same direction as aware subjects indicting these processes may occur outside of the conscious awareness. Emotional responses to the stimuli, recorded at the end of the experiment, also varied as a function of how well the cue predicted the outcome. Thus pleasantness was greatest for AX, then BX, and rated lowest for CX, and the opposite pattern observed for anxiety towards the stimuli. More critically, in the condition in which the reward value was not manipulated the number of instrumental responses performed increased linearly in accordance with the expectancies of the outcome (AX>BX>CX), thus driven by the explicit knowledge/prediction of the outcome, but this effect was abolished in both devaluation and revaluation experimental conditions despite expectancy responses remaining intact. When participants were experienced with the revalued condition, instrumental responding was elevated across all stimuli, but when the reward was devalued the instrumental performance was markedly reduced. Taking together the intact expectancy ratings and altered

instrumental responding, I concluded that the instrumental performance effect relies on the current reward value to drive motivated seeking behaviour and that this can be dissociated from the knowledge of the outcome. It is important to note that the effect on instrumental performance was observed in the first half of transfer trials, and in the second half the effect was abolished even in the control condition, most likely driven by participants acquiring the new contingencies as the transfer test was performed under nominal extinction.

The purpose of paper 3, described in chapter 5, was to investigate the interaction between positive mood and incentive salience of conditioned stimuli. As the effect of negative mood on instrumental responding could be likened to that of reward devaluation, bringing about a reduced instrumental responding despite intact attention and expectancy, it was proposed that positive mood may bring about similar effects to that of the revalued condition in paper 2. The experimental design utilised in paper 3 was the same procedure adopted in paper 1 (study 2) whereby the stimuli predicted an aversive outcome. A mood induction procedure was also carried out as per the design of paper 1 (study 2) but inducing state positive or neutral mood. As found in the previous experiments, responses produced by aware participants for both expectancy ratings and observing times for each stimuli were matched for both experimental groups during Pavlovian learning and transfer. Expectancy ratings were also again maintained into the transfer test by aware participants and the prediction error hypothesis of attention was again supported with observing times for BX being greatest during Pavlovian learning and lowest during transfer when compared to AX and CX. Furthermore, explicit knowledge/prediction of the outcome drove the transfer effect, such that in the neutral mood condition the instrumental performance to avoid the outcome increased linearly in accordance with the expectancies of the outcome (i.e. AX>BX>CX). However, in

contradiction to our hypothesis, positive mood did not increase instrumental responding above that seen in the neutral mood condition, nor did it eliminate the transfer effect. A suppression in the transfer effect was observed when participants were experienced with induced positive mood, driven mainly by an overall increase in behavioural output for stimulus CX. As observed with induced negative mood the impact of state positive mood on instrumental responding was dissociated from attention and expectancy. Although it is possible that positive mood manipulated instrumental responding for stimulus CX by driving motivation to avoid the aversive outcome under any circumstance (i.e. even in the presence of stimulus CX associated with absence of the outcome), it is also possible that the reduced instrumental discrimination between stimuli is a result of acquiring the new contingencies more quickly than those in the neutral mood condition. It is unclear by which mechanism the positive mood acted and in order to determine if the new contingencies had been acquired more quickly by those under induced positive mood state, a study investigating the effect of state mood on learning was required.

The results from paper 3 indicated that positive mood may have facilitated quicker acquisition of novel contingencies (as transfer was performed under nominal extinction), thus, it is plausible that certain state moods may accelerate learning and this may also occur during Pavlovian conditioning. The purpose of paper 4, described in chapter 6, was to test this hypothesis by adding additional trials to a Pavlovian discriminative learning procedure whereby three compound stimuli were predictive of a reward with a 90%, 50% and 10% probability as per the other designs used in earlier experiments (described in chapters 3, 4 and 5). Prior to the Pavlovian learning task the mood induction procedure was completed to induce state positive, negative or neutral mood. By adding additional trials to the Pavlovian design this would increase the

likelihood that all participants able to acquire the contingencies would become aware. Results indicated that those participants experienced with negative mood took longer than those under induced positive mood to become aware of the outcome contingencies and successfully discriminate between the stimuli using the expectancy response. The use of an eye-tracker was also adopted in this study to improve the depth of information gained regarding attention to the stimuli under different mood conditions during learning. Papers 1 and 3 (described in chapters 3 and 5) demonstrated no difference in observation times toward the stimuli between mood conditions, albeit in these experiments mood was only altered for the transfer stage, but as discussed for paper 3 during this stage learning may occur differentiating the experimental groups. Paper 2 (described in chapter 4) also demonstrated acquisition of the extinguished outcome contingencies by all participants during the second half of the transfer stage of the experiment. Mood state disrupted observational patterns to the stimuli such that induced positive and negative mood states reduced overall observation time in BX and CX trials, as well those in a induced negative mood state only showing an increased bias for stimulus C over X; interestingly, those under neutral and positive induced mood showed an attentional bias for stimulus A over X, but not C over X. Average pupil size in all trial types was increased by positive mood. I concluded that induced positive and negative mood states differentially affected emotional and observational responses to Pavlovian stimuli predictive of reward, and also impacted on the rate of acquiring contingency knowledge of the same predictive stimuli. However, I also acknowledged that as a stand-alone study, in the absence of further similar work, the precise mechanisms driving these changes remained unclear.

In summary, the conditioned incentive properties of the stimuli were mediated by state mood for both appetitive and aversive outcomes. However, the effect observed was

different for aversive and appetitive outcomes, and also dependent upon the type of state mood induced (positive or negative state mood). Under conditions when Pavlovian predictive contingencies were first learnt, and then a mood state induced, the motivation to perform an instrumental response for the outcome was altered and so was the discriminative instrumental responding during PIT. When the outcome was a reward, induced negative mood reduced the motivation to perform the response, despite expectancy of the outcome remaining, and abolished the PIT effect, in much the same way as when the reward outcome was devalued. Therefore negative mood may have served to alter the perceived value of the reward. In contrast, when the outcome was aversive, the PIT effect remained intact but negative mood did reduce the number of responses, indicating a reduction in motivation, but not loss of discriminative value of the outcome. These effects on motivation (altered PIT response) were dissociated from attention and expectancy of the outcome, which remained intact. The results also demonstrated a difference between reward and punishment prediction when under induced negative mood. When motivation was challenged under induced positive mood again dissociation between expectancy and attention versus motivation was observed. Whilst attention and expectancy remained intact into the transfer test, the PIT effect was altered, but not abolished. Expedited learning of the extinguished contingencies may be the cause but this is unclear. It was only when an induced mood state was challenged in a learning paradigm that reduced and increased speeds of acquiring novel contingencies regarding stimuli and predicted outcomes by state negative and positive mood respectively could be more clearly observed. The discussion will begin with an attempt to clarify the mechanisms underlying attention in PIT, and also the motivational mechanisms underlying the ability of stimuli to drive reward-seeking behaviour in the same model. The impact of positive and negative state mood on motivation and learning

will then be discussed and the possibility of differences in aversive and reward outcomes will be addressed. Potential concerns regarding the methodologies in the experiments will be reported, followed by implications of this research and suggestions for future research, which will end the discussion.

Mechanisms of attention involved in PIT

An overview of the findings indicated that in our general PIT design attention was driven by the prediction error theory of attention (Pearce & Hall, 1980), as opposed to the incentive theory of attention (Bindra, 1969). In papers 1, 2 and 3, attention, as indexed by observing times (time spent viewing the stimuli), was driven by uncertainty and this was unaffected by the manipulations of mood or reward value in these studies. Furthermore, this could be linked to expectancy of the outcome as this was also unchanged by experimental manipulations of mood or reward value. By contrast, motivation was driven by expectation of the outcome, but this is discussed later. In Pavlovian training, observing times were greatest for the uncertain predictor compound BX compared to the reliable predictor (AX) and non-predictor (CX) compounds, supporting previous human (Hogarth, Dickinson, et al., 2008; Trick, et al., 2011) and animal data (Collins & Pearce, 1985; Kaye & Pearce, 1984) which reported decreased attention for certain consistent predictors whilst maintaining attention for partial predictors, and also demonstrating that the observing response can be used to index the uncertainty associated with a CS (Hogarth, Dickinson, & Duka, 2009). In further support, when the Pavlovian contingencies were altered from training to transfer, observing times reversed their relative magnitude, becoming smaller for stimulus BX compared to AX and CX. This effect might be attributed to differential discrepancies

between the expected probability of the outcome in each stimulus, as learnt in Pavlovian training, relative to the actual probability in the transfer test which was carried out under nominal extinction, i.e., prediction error. Specifically, over-expectation of the outcome in stimulus AX may have generated a large negative prediction error, whereas under-expectation of the outcome in stimulus CX may have generated a large positive prediction, compared to BX, which retained its original partial contingency and so was associated with the same moderate prediction error as was seen in Pavlovian training.

The finding in paper 2 (described in chapter 4) that even participants who were unaware of the contingencies, based on expectancy ratings, showed a significant modulation of observing time in the transfer test in the same direction as aware subjects, accords with the view that observing time is associated with the early stages of learning. Our criterion for distinguishing aware and unaware participants indicated that the unaware group were quantitatively less knowledgeable of the predictive contingencies as opposed to being completely unaware, and formed the basis of our decision to include extended Pavlovian learning trials in paper 4 (described in chapter 6) as mentioned earlier. The finding that observing time increased for AX and CX relative to BX when the contingencies changed in transfer suggests that this reallocation of attentional resources is the first response domain to reflect the early formation of predictive knowledge; this is also interesting with regard to paper 3 (described in chapter 5) where it was suggested that the modulation of instrumental responding by those under induced state positive mood could be apportioned to acquisition of the novel contingencies, however their observing times in the transfer stage were not significantly different to that of participants in the neutral mood condition. It is, however, not possible to discount this theory as those under neutral mood may also have started to gain new predictive knowledge. To address this issue, the data from paper 4 (described in chapter 6)

demonstrated how those under induced neutral and positive state mood showed expedited learning when compared to participants in the negative mood condition, and they also showed the same patterns of attention (whereby observing times were in accordance with the theory of prediction error) which again differed to the negative mood condition. This provides support for the proposition that induced positive mood facilitates learning, when compared to negative mood, but is by no means conclusive, and is discussed in more detail later. To conclude, attention in the general PIT model used here was driven by prediction error and can be dissociated from motivation, but can, to some extent, be utilised to index learning.

Mechanisms facilitating the ability of stimuli to drive reward seeking behaviour

As discussed earlier instrumental responding in the presence of conditioned stimuli is indicative of activation of an appetitive motivational system by the stimulus and can be modelled experimentally using PIT. Such motivated instrumental responding can be brought about by a specific representation of the reward and drive the responding through specific outcome expectancy, or it could reflect activation of a central motivational system and drive the instrumental responding more generally. PIT is one method by which outcome-specific and general motivational systems have been separated. The PIT design utilised in papers 1, 2 and 3 (described in chapters 3, 4 and 5) was shown to be susceptible to an outcome devaluation procedure in paper 2 (described in chapter 3), indicating performance to be goal-directed and most likely allowed for devaluation because the reduction in goal-value rendered it inadequate to motivate the instrumental behaviour. Traditionally, it was thought that PIT was not mediated by the expected incentive value of the outcome (Rescorla, 1994a), however, other recent advances have also demonstrated devaluation in certain PIT models (Allman, et al.,

2010). In the PIT procedure utilised in this thesis, the cues predicted the outcome with different levels of uncertainty (90%, 50% or 10%) and performance was activated based in accordance with the expectancy of the outcome. Therefore, it may be that such predictive uncertainty during Pavlovian conditioning is a key determinant of the behavioural consequences of associative learning, and that the PIT effect is related to explicit knowledge of the outcome. Thus the discrepancies in the literature regarding the ability of devaluation techniques to modulate PIT, as discussed in chapter 1, could be explained by the method in which the PIT procedure was run and the reinforcers selected.

In paper 2 (described in chapter 4) expectancies of the reward remained unchanged after devaluation/revaluation manipulations and attention remained intact, despite suppression of instrumental responding, it therefore seems that the current value of the reward modulates instrumental performance for the outcome. Therefore, taken alongside the results of study 1 (described in chapter 3) it may indicate that an induced negative mood state interacts with motivational processes by rendering the outcome less valuable to the agent and thus reducing the motivated behaviour to obtain that outcome, and this is discussed in more detail below. As appetitive and aversive outcomes are dissociable with regard to the impact of induced negative mood it is difficult to determine if this theory would also be valid when the outcome is aversive.

The implications of state mood on learning and underlying mechanisms

In paper 4 (described in chapter 6) the results demonstrated that those under induced negative mood took longer to demonstrate successful discrimination of the predictive nature of the stimuli. The three Pavlovian stimuli (AX, BX and CX) predicted reward

on 90%, 50% and 10% of trials respectively, and an extended number of trials were administered. Interestingly, the same percentage of participants assigned to each mood condition ultimately became aware. The pattern of learning (indexed using expectancy ratings) and attention (indexed using observing times) was similar for participants experienced with neutral and positive mood, which differed from those under induced negative mood. This finding is important, because attention has been shown to correspond to learning as observing times adapt to the evolving contingencies associated with stimuli, both in the data in this thesis whereby observing times switch between Pavlovian learning and transfer when outcome contingencies were altered and also previously (Trick, et al., 2011) where the same results were found. Indeed, this supports the claim that positive mood facilitates learning, when stimuli are predictive of either aversive (paper 3 – chapter 5) or appetitive (paper 4 – chapter 6) outcomes. In contrast, those under induced negative mood state demonstrated slower learning, and this is an interesting finding since the concept of depressive realism describes the theory whereby participants in depressive states have more accurate judgements of contingency outcomes (Alloy & Abramson, 1979), which would perhaps indicate a higher rate of learning should be experienced. However, the same theory also describes that those in a non-depressive state (i.e. positive and/or neutral induced state mood conditions in the experiments in this thesis) would overestimate the degree of contingency between their responses and outcomes thus facilitating correct expectancy responses as they may overestimate the 90% stimuli to 100%, and the 10% to 0%; this mechanism would also explain the attentional processes seen as these same participants would apportion minimal attention to the 90% and 10% stimuli as they might deem them “certain” which accords with the data collected in this thesis. One important caveat to this is that depressive realism may rely on trait mood, as opposed to state, which is what was

challenged in the experiments detailed in this thesis. As previously mentioned the impact of BDI, which is commonly used to assess trait depressive mood (Allan, Siegel, & Hannah, 2007) will be addressed later.

There are several possible explanations for the reduced rate of learning seen in the individuals in the negative mood condition in paper 4 (described in chapter 6). Firstly, as the data for the neutral and positive mood conditions showed a gaze bias for the informative stimulus A, over the common X stimulus, which was larger than the bias towards B over the concurrently presented X but this effect was abolished in the negative mood condition, and replaced by a gaze preference towards the non-predictive stimulus C, over the concurrently presented X stimulus, in CX trials. This indicates a bias in the negative mood condition for the aversive stimulus (stimulus C - which may be viewed as a punishment stimulus), supporting previous data associating negative mood with increased punishment prediction but not reward prediction (Cools, Robinson, et al., 2008; Rogers, et al., 2003) as paper 4 utilised a reward outcome. A reduced discrimination between expected gains has also been demonstrated previously (Rogers, et al., 2003) which may also account for the delayed learning in the negative mood condition in the study presented in this thesis. With regard to the finding that those under induced negative mood took longer to acquire the contingency knowledge, this does correlate with some previous findings that those in a negative mood state show reduced learning (Chase, et al., 2010). It is worthwhile to note, however, that the negative mood group in the aforementioned study was confounded by high BDI scores, but this was not the case in our study whereby the conditions were matched for BDI scores and this is addressed later. Additionally, a suppressed motivation is the most commonly proposed explanation for reduced learning under negative mood, and it is possible that attenuated learning reflects decreased confidence. This is particularly

likely as our study required the participants to make explicit responses indicative of learning, thus the reduced speed of learning may be driven by a reduction in confidence to provide the correct response to the expectancy-rating question, which may be linked to attenuated impulsivity in the negative mood condition. This link to reduced impulsivity is unlikely, however, when taking into account result(s) in certain previous literature (Mobini, et al., 2000) as described earlier.

Negative mood and reduced motivation (Cools, et al., 2005) have long been linked and therefore it seems this is a likely explanation and is discussed in detail in a separate section. In further support, recent advances in the study of attention have demonstrated a link between action control and attentional processes (Bekkering & Neggers, 2002; Wykowska, Schubo, & Hommel, 2009). More specifically, it has been shown that sensitisation of the perceptual system to information about guiding a certain action is elicited by the preparation to perform that same action (Wykowska, et al., 2009). Recent expansions in this field also propose motivation as a driver for visual attention and have introduced pupil size as an indirect measure of motivation/effort (Wykowska, Anderl, Schubo, & Hommel, 2013). This is interesting with regard to the data presented in paper 4 (described in chapter 6) as an increased average pupil size in the positive mood condition across all trial types compared to the negative and neutral mood conditions was observed. The increased pupil size in the positive mood condition was accompanied by an increased attentional and behavioural preference for AX trials at an early stage of the experimental procedure and may have also driven the faster learning in the positive mood group, as those under induced positive mood also acquired contingency knowledge earlier in the same study. Hence the data support the suggestion that those under induced negative mood are less motivated than those under positive mood, which

would impact learning in a Pavlovian contingency design such as that used experimentally in this thesis.

The implications of state mood on motivation and underlying mechanisms

Induced state positive mood appeared to facilitate expedited learning encompassing the results on attention and expectancy in this thesis, and this potentiated learning was seemingly responsible for the changes observed on the PIT effect by those experienced with positive mood. However, data from negative mood manipulations appeared to indicate the impact on the PIT effect observed (by those in the negative mood condition) was driven by motivational influences, and was less clearly defined. In particular, when under induced negative mood, the mechanisms underlying the effects on Pavlovian discrimination learning and instrumental transfer are not isolated to one particular effect, however the results seen using a induced positive mood state seem more defined and appear to be predominantly modulated by increased speed of learning.

One point that does require clarification regarding positive mood, is the effect of induced positive mood on the transfer effect in paper 3 (described in chapter 5) which utilised aversive outcomes. It was anticipated that positive mood would increase instrumental responding, but this was only observed in the presence of compound stimulus CX and the PIT effect remained intact (such that responding corresponded to the predictive nature of the stimuli: AX>BX>CX). One possible explanation is that as the outcome was aversive, and as mood in general has been shown to be linked to punishment prediction (which will be discussed in more detail below), the PIT effect remained intact as extreme mood has been related to enhanced punishment prediction. In opposition to this, it is possible that given further trials in which the outcomes were

continually extinguished, the instrumental responses for stimulus AX would be reduced, and for CX would be increased further still, to abolish the PIT effect and demonstrate learning of the new contingencies (as all stimuli had become 50% contingent with the outcome). This is a speculative explanation and would require further study to clarify, but it seems possible given the observed effects of induced positive mood on learning. In any case, the results demonstrated, for those under induced positive mood, a reduced PIT effect driven by an increased number of instrumental responses being made during CX trials; this is either a demonstration of novel learning or increased motivation to avoid the response under any circumstance (i.e. even in the presence of the non-predictive CX stimulus). In contrast, the effects of induced negative mood can be more strongly linked to motivation.

It is important to try to dissect the processes by which motivation is modulated by negative mood in the PIT studies utilised in this thesis, in particular the experiments in paper 1 (described in chapter 3). The results demonstrated a dissociation between attention (driven by uncertainty) and expectancy (knowledge of stimulus contingencies) versus behaviour (driven by prediction), when the outcomes were either appetitive or aversive. Although the PIT effect was modulated by induced negative mood for both outcomes the impact was different. For both outcomes the number of instrumental responses made was reduced, but when the outcome was punishment the PIT effect remained, and when the outcome was reward the PIT effect was abolished. Taken independently, the effect observed when the outcome was reward is in accordance with data from the devaluation study in paper 2 (described in chapter 4), indicating that the perceived reward may have been devalued by the induced state mood thus reducing the motivation to perform the instrumental response. As a reduction in responses to all three compound stimuli in the appetitive design was observed this indicates that novel

learning has not taken place, and that an impact on the reward value is likely. Previous data (Rogers, et al., 2003) suggests that those under a state of negative mood, albeit induced by acute tryptophan depletion, do show a reduced discrimination between expected gains, which would accord with the reward becoming devalued. The same study also demonstrated little support for an impact on discrimination between expected losses, which again accords with the data in paper 1 (described in chapter 3), as the PIT effect was not abolished when the outcome was aversive and the reduced responses could be accounted for by general motivational mechanisms. It is also possible that the impact of an induced negative mood state on expected reward may be driven by depressive realism mechanisms, as discussed earlier, which could account for the altered perception of the reward driving a reduction in motivation. Anhedonia could also account for the modulation in reward perception, as it is associated with diminished interest in pleasurable events, such as reward (Der-Avakian & Markou, 2012; Huprich, 2013) and therefore could conceivably lead to a reduced motivation to perform an instrumental response to obtain that reward or avoid aversive outcomes.

The reduction in motivation to perform the instrumental response could also be driven by a general reduction in impulsivity. Negative mood, as modulated by serotonin, has consistently been linked to impulsive behaviours (e.g. Cools, Roberts, et al., 2008; Cools, Robinson, et al., 2008; Roiser et al., 2006, etc), but the exact contribution is often unclear (Anderson, et al., 2003; Ho, Al-Zahrani, Al-Ruwaitea, Bradshaw, & Szabadi, 1998). It is therefore possible that negative mood is linked to a decrease in motivation more generally. Thus, motivation is reduced when the outcome is to avoid punishment and also to gain reward in the natural environment, but is dissociated from the expectancy knowledge of that same reward, and also prediction error. The results in paper 1, study 1, (described in chapter 3) indicate that the observed reduction in

motivation is driven by an attenuated sensitivity to the reward itself, such that the reward is devalued by the induced negative mood state, and not a reduction of the impact of the conditioned signals as knowledge of the S-O contingencies were maintained (Rogers, et al., 2003). The reduction in motivation to gain reward therefore provides an insight into treating drug addiction (Koob, Sanna, & Bloom, 1998) and is also relevant for the link between depression and cognitive impairment. The data may also demonstrate a possible link between current mood state and reward value.

In addition, the results in paper 1 (described in chapter 3) are in accordance with one conclusion drawn in previous studies (Dayan & Huys, 2008; Rogers, et al., 2003) that negative mood does not affect discrimination between expected outcomes when they are aversive but may drive a reduction in general motivation to seek reward/avoid punishment by altering the way in which reward cues are processed. It has also been demonstrated previously that an enhancement in punishment prediction is associated with negative mood, but not reward prediction (Cools, Robinson, et al., 2008). This dissociation is interesting with regard to the data in paper 1, as in study 1, where a reward outcome was utilised, the number of instrumental responses performed for each set of stimuli to gain the reward was reduced and the PIT effect (responses AX>BX>CX) was abolished. However, in study 2 when the stimuli were predictive of an aversive outcome the response to avoid this outcome was reduced but the PIT effect was maintained. This is also in agreement with certain theories that hypothesise serotonin (reduced under tryptophan depletion procedures) is involved in punishment prediction, but not reward (Daw, Kakade, & Dayan, 2002). Further to this link between aversive motivational processes with serotonin and negative mood, dopaminergic systems have been shown to work in an opposing model and be linked with appetitive motivational processes.

In summary, taking into account the evidence available I conclude that the effects observed in this thesis of positive mood are driven by enhanced learning in both aversive and appetitive experimental designs. In contrast, utilising the data in this thesis and previous literature, I conclude that the effects observed of induced negative mood are more likely driven by motivational mechanisms and the effects seen in aversive and appetitive models are distinct from one another.

General limitations and methodological concerns

One major methodological concern is that the model used may not have reflected a naturalistic setting. In particular, the expectancy question may have directed attention, biased goal-directed behaviour and driven motivation. Likewise, it is also possible that emotional questions regarding the stimuli may have influenced behaviour and attention towards the stimuli. However, the emotional questions were limited to the end of the experimental session and therefore would not have been able to impact upon the results. In addition, as the results of the expectancy question we were required to determine awareness of the outcome contingencies they formed a vital section of the studies and could not be eliminated. Indeed, the motivational responding in some circumstances was dissociated from the results of the expectancy questions thus it seems they did not influence the behaviour.

Another issue related to the impact of state mood versus trait mood in papers 1, 3 and 4 (chapters 3, 5 and 6). This thesis aimed at investigating the impact of state mood. Previous data (H. W. Chase, et al., 2010) has implicated BDI in manipulating behavioural responses, and BDI scores are indicative of trait depressive mood. However, all experimental groups (in all studies presented in this thesis) were matched

for BDI scores in an effort to eliminate any potential impact of trait depressive mood. Further to this, a VAS was used to index the outcome of the MIP and indicated state moods were successfully induced. However, it may be beneficial in future studies to attempt to derive a biological marker for this same purpose. The impact of this may be negated to some extent as previous studies have adopted the use of certain biological markers, which have then been dissociated from the behavioural effects observed. For example, salivary cortisol has been tested as a potential marker but has produced mixed and potentially misleading results (L. Clark, et al., 2001; Vielhaber et al., 2005). In addition, the data presented in this thesis are in contradiction with certain examples demonstrating a role of damaged serotonergic pathways in increasing reinforcer value (i.e. (Wogar, et al., 1991)), however this may be due to a distinction between an induced negative mood state and alteration of serotonin pathways, as well a difference in human and animal models.

Furthermore, reduced serotonin, which can be brought about by acute tryptophan depletion, is involved in the development of negative mood and has also been linked to increased impulsivity (Anderson, et al., 2003; Ho, et al., 1998). Although we did not directly measure impulsivity, the data presented in this thesis demonstrate a general reduction in motivation when under induced negative mood, which may imply a reduction in impulsive behaviours and a potential contradiction to the aforementioned study. However, serotonin manipulations and induced negative mood may bring about differential behavioural changes which could account for these differences. In addition, the data presented in this thesis also highlighted a dissociation between expectancy and the motivational influences of negative mood. Expectancy may therefore be associated with impulsive actions but not the motivational affects. This separation is difficult to dissect in the model utilised.

Finally, although I have claimed that the switch in observing times in the learning stage versus the transfer stage of the PIT model (AX and CX < BX during learning, versus, AX and CX > BX during transfer) is due to prediction error it may also be due to stimulus redundancy. Therefore, attention as supported by the prediction error theory may be impacted by the partial predictor (stimulus compound "BX") not being a truly uncertain stimulus and may have been deemed redundant by the experimental participants. However, in some instances during the studies presented in this thesis participants preferentially attended to the BX stimulus and certain learning theories state that redundant stimuli will become ignored (Kruschke & Blair, 2000). It is not possible to fully distinguish between stimulus redundancy and prediction error using the studies presented in this thesis, and further study is required to clarify this distinction.

Implications

The strongest implication of the series of studies in this thesis is that induced negative mood reduces the general motivation to perform an instrumental response to gain reward and to avoid punishment, and that this process can be dissociated from explicit knowledge about, and attention to, stimuli predictive of the outcome. In the context of addiction this may provide a possible pathway for intervention in accordance with motivation theories (Robinson & Berridge, 2003) of addiction and relapse. Furthermore, within the context of negative mood the effects on punishment and reward prediction differ in the presented studies, in support of previous literature (Cools, Robinson, et al., 2008; Rogers, et al., 2003). These data therefore provide additional support for the motivational and neurobiological interactions between negative mood and instrumental responding; such as, the opposing effects of dopaminergic and serotonergic mechanisms of motivation (Cools, Roberts, et al., 2008) and the link between negative mood and

punishment, but not reward, prediction (Cools, Robinson, et al., 2008). In a similar vein, induced negative mood was also dissociated from positive mood with regard to the impact on learning, such that positive mood facilitates potentiated learning. The different contexts under which induced state negative and positive mood are able to manipulate learning and the PIT effect may guide interventions in a more precise way.

Future directions

While the present findings indicate that levels of motivated behavior may reflect the current state mood of an individual, and may therefore be implicated in abnormal and undesirable behaviors, further investigations are required in order to establish this connection. A major problem with making inferences from the current data is that learning and motivation may not be mediated in the same way for individuals with drug addictions or other relevant clinical disorders. Indeed, the majority of other studies investigating the impact of negative mood utilized acute tryptophan depletion in healthy subjects to bring about a biological state of reduced serotonin, however this may also not provide a full picture and often required the use of MIPs to successfully induce a state mood (Robinson & Sahakian, 2009b). The exact mechanisms by which state mood interacts with learning and motivation in clinical populations requires further investigation and this clearly has implications for the use of induced mood in interventions for addictive behaviors, among other conditions. For example, the application of mood interventions to populations of drug users requires exploration.

A second direction, equally relevant, is the impact of positive and negative mood on acquisition of contingency knowledge, when the outcome is aversive, over a period of extended Pavlovian learning. The data presented in this thesis, taken together with

additional literature (Cools, Robinson, et al., 2008; Rogers, et al., 2003) indicate a state of negative mood enhances punishment prediction and therefore it would be pertinent to investigate this effect further in order to link these findings to clinical populations and intervention strategies. Results indicated induced negative mood reduced speeds of learning in comparison to induced positive mood when the outcome was appetitive, but it is not clear if this would be the case if the outcomes were aversive. In a similar vein, the impact of positive mood on motivation to perform an instrumental response when the outcome is appetitive is also an interesting direction of future study. The findings in this thesis indicated induced positive mood facilitates learning and acquisition of novel contingencies, but this was limited to aversive outcomes in the PIT design.

The data presented in paper 4 (described in chapter 6) showed an increased average pupil size in the positive mood condition across all trial types when compared to the negative and neutral mood conditions. In addition, previous studies have also demonstrated a difference in pupil size when under certain conditions between anxious patients and controls (Bakes, Bradshaw, & Szabadi, 1990) and also a correlation between state anxiety ratings associated with punishment and pupillary light reflexes (Bitsios, Szabadi, & Bradshaw, 2002). Albeit these results link anxiety to pupil size, there are recognised similarities between anxiety and negative mood and further study into the impact of negative mood on pupillary reflexes may well be a fruitful avenue of investigation. Pupil size may also provide a useful physiological marker for future mood induction studies.

Concluding remarks

In this thesis, the mechanisms underlying the impact of state mood on learning, and more specifically motivation to perform an instrumental response were considered. I suggested that negative mood was an important factor in reward seeking, and would be implicated in the perception of outcome value, although it was acknowledged that the response to reward and aversive outcomes might differ.

Using classical conditioning procedures I demonstrated that induced positive mood facilitated learning, whist induced negative mood induced attenuated speeds of acquisition of predictive contingencies. Further to this finding, this effect of increased learning when under positive mood was also observed into the transfer stage of PIT. Additionally, I established that motivational mechanisms were at the forefront of the impact of induced negative mood on the PIT effect, and that the response to aversive and appetitive outcomes was modulated by independent mechanisms when under an induced negative mood state. Reward outcomes were devalued by negative mood, and thus the motivation to respond in the presence of such predictive stimuli reduced, and the PIT effect abolished; whereas, the responses to avoid punishment were reduced most likely by attenuated motivation, but the presence of enhanced punishment prediction rendered the PIT effect more stable. Finally, there was also a clear indication that attention, driven by uncertainty in this paradigm, and knowledge of S-O contingencies, were not affected by state mood and were therefore separated from instrumental responding. These results demonstrate that whilst attention and contingency knowledge are important in modulating learning and motivated behavior, the impact of state mood is also a key factor in facilitating these processes and may, under appropriate circumstance, influence learning and motivation.

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Chapter 3

Paper 1: Motivational mechanisms underlying the effect of negative mood on Pavlovian-to-Instrumental transfer.

Abstract

Mood states have been suggested to affect a range of cognitive and other behavioural processes. To study the effects of negative mood on a motivated response to gain reward or avoided punishment, a Pavlovian-to-instrumental transfer (PIT) model was used. This model has been extensively studied in animals and has, more recently, been adapted for use in humans. It has been demonstrated that humans are able to transfer predictive Pavlovian stimulus-outcome relationships to independently learned instrumental responding for that same outcome and it is believed that such a transfer effect is modulated by the predictive strength of the stimulus, which, in turn, exerts a motivational influence driving the instrumental responding. The effect of negative mood on the likelihood and vigour of this instrumental response, in humans, is examined in two general PIT studies in the presence of positive or aversive outcomes. A Pavlovian training schedule was used in which three compound stimuli AX, BX, CX predicted an outcome of reward (10p) or an aversive noise on 90%, 50% and 10% of presentations respectively. This was followed by a mood induction procedure, a short phase of instrumental training and finally, transfer performed under nominal extinction. During transfer, contingency awareness and emotional ratings were unaffected by the mood induction procedure. However, instrumental responding was reduced in the negative mood condition. These findings suggest that the motivation to perform an instrumental response to obtain reward, or to avoid punishment, in the presence of predictive stimuli is reduced under conditions of negative mood which could help our understanding of the effects of mood state on cognitive performance in patients suffering from mood

disorders, and may also highlight a role that negative mood plays on addictive processes.

Key words: learning, human, motivation, negative mood, addiction, attention.

Introduction

The functional role(s) of mood in learned and motivational behaviours is the subject of much debate; indeed previous studies investigating these effects have shown there to be an implication of mood on factors believed to be involved in Pavlovian-to-instrumental transfer (PIT) including impulsivity (Clark, et al., 2005), instrumental learning and information processing (Finger, et al., 2007; Merens, et al., 2007), motivation (Cools, et al., 2005) as well as emotional and behavioural processing (Cools, Roberts, et al., 2008). Addiction is also linked to these factors and mood may, therefore, also have a link with addictive processes (Cools, et al., 2005) and PIT is a model that has often been used to study addiction (Everitt & Robbins, 2005). It has been widely accepted that humans are able to transfer the predictive Pavlovian stimulus-outcome relationships to independently learned instrumental responding for that same outcome (e.g. Balleine & O'Doherty, 2009; Hogarth, Dickinson, Hutton, Bamborough, et al., 2006, etc) [for a review, see Lovibond & Shanks (2002)] and it is believed that such a transfer effect is modulated by the predictive strength of the stimulus, which, in turn, exerts a motivational influence driving the instrumental responding (Everitt & Robbins, 2005). Motivation is an important response to stimuli, and reward, required for many normal processes (Robinson & Berridge, 2003) but is also implicated in abnormal processing and driving of certain behaviours, so it will be interesting to observe the motivational response to gain reward, or avoid punishment, exerted by those under negative mood. It is interesting to note that in our hands devaluing the rewarded outcome can disrupt the transfer effect and may support the claim that the transfer effect (perhaps by decreasing motivation to gain, or influence, that outcome) relies on the current value of the reward as well as the ability to predict the outcome. This disruption may be replicated under conditions of negative mood, but the causal factor may be different.

Negative mood, e.g., in depressive disorders or under conditions of drug withdrawal, is often associated with anhedonia, characterised by decreased sensitivity to pleasurable events (Rogers, et al., 2003), which in turn may affect cognitive performance due to a change in the predictive strength of a stimulus and hence contribute to cognitive deficits associated with these disorders. PIT is mediated by the prediction of an outcome (which may be viewed as a pleasurable event when the outcome is a reward, or cessation of an aversive outcome) such that the magnitude of the transfer effect observed in PIT accords with the associative (or predictive) strength of the stimulus (Balleine & Ostlund, 2007). In the present study we were therefore particularly interested in the extent to which induced negative mood might reduce instrumental responding in a PIT paradigm and whether this reduction would accord with the predictive strength of the stimulus or eliminate responding entirely. It is also not clear that negative mood will model anhedonia, however, it is generally accepted that by manipulating serotonin and/or by carrying out mood induction procedures that the effects of mood may be studied (Cools, et al., 2005). Therefore, we predicted that negative mood would reduce the value of the outcome by inducing anhedonia, which would in turn lead to a reduction in instrumental responding. Two complementary studies were carried out aiming to investigate the transfer effect under conditions of negative and neutral induced mood, using positive (study 1) and aversive (study 2) outcomes.

The objective of the current experiments was, therefore, to study under which circumstances the motivation to seek reward may be challenged and to determine whether state negative mood may manipulate motivation, and if a dissociation between avoiding punishment and obtaining reward would be seen. Additionally, we were interested in investigating the methods by which motivation might be affected by negative mood as it has been previously demonstrated that serotonergic manipulation

together with negative mood, affects the motivational properties of stimuli predictive of rewards (Cools, et al., 2005) and whether state mood could be utilised in potential treatments for addiction. A Pavlovian training schedule was used in which three compound stimuli AX, BX, CX predicted an appetitive reward (study 1) of 10p or a aversive noise (study 2), on 90%, 50% and 10% of presentations respectively (Hogarth, Dickinson, et al., 2008). After Pavlovian training had been completed a visual (Smith, et al., 2005) and musical mood induction procedure, adapted from one previously described in Robinson, et al., (2009) was carried out. In the transfer stage that followed, under nominal extinction, the ability of the stimuli to elicit the instrumental response was determined to study the PIT effect. In addition, participants were able on each trial, throughout all stages, to view the stimuli for an infinite duration as determined by holding down a key; this observation time was used as a measure of attention towards each set of compound stimuli. It is important to note that predictive uncertainty is also able to attract attention under certain conditions (Trick, et al., 2011). It is anticipated that observation time will be proportional to the uncertainty of the stimuli, such that greatest attention will be paid to BX compared to the certain compounds AX and CX. In contrast, it is believed that the transfer effect will be AX>BX>CX and that instrumental responding will be reduced under conditions of negative mood.

Procedures were approved by the University of Sussex Ethics Committee. Participants were informed they were allowed to withdraw at any time.

Materials and Methods

Participants

Healthy subjects who were taking no medication, as determined by a medical health questionnaire, were recruited. Ethical approval was obtained from the University of Sussex ethics committee and all subjects gave written informed consent prior to participation; all participants were fully debriefed at the end of the session and compensated for their time with £15.

Study 1

Fifty-three subjects (29 males) took part in the study, 8 females and 13 males were excluded due to failure to successfully learn stimulus-outcome contingencies. The remaining thirty-two subjects, deemed "aware", were divided into neutral mood (n = 16; 8 females) and negative mood (n = 16; 8 females) conditions. The mean age of subjects was [years] 22.5.

Study 2

Forty-four (19 males) took part in the study, 8 females and 4 males were excluded due to failure to successfully learn stimulus-outcome contingencies. The remaining thirty-two subjects, deemed "aware", were divided into neutral mood (n = 16; 8 females) and negative mood (n = 16; 9 females) as for study 1. The mean age of subjects was [years] 21.4.

Experimental procedure

Subjects were asked to attend the laboratory on one occasion. They were instructed to abstain from alcohol for twelve hours prior to the testing session. In addition they were asked to avoid consuming anything high in caffeine immediately before the test session.

Participants were informed they would be compensated for their time as well as receiving any money they won on the task.

Materials

The task was presented on a 20" Dell P1130 monitor (Dell Inc, Berkshire, UK) and programmed using E-prime v1.1 software (Psychology Software Tools Inc.; Pittsburgh, PA). The 4 visual stimuli used (see figure 1) were black patterns displayed on a grey background, 10.2cm squared at a resolution of 1280 x 1024.

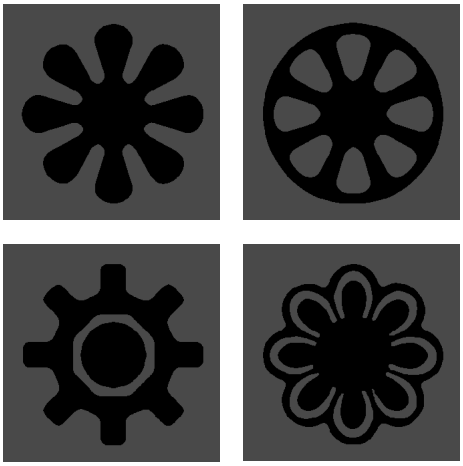


Figure 1: *Materials used during the computerised task throughout the PIT procedure - the four visual stimuli assigned to A, B, C and X in a counterbalanced fashion*

Text font and size was Times New Roman 25pt. The auditory music (for the mood induction procedure and aversive noise) was played through Sennheiser PX200 headphones. Participant responses were collected via a Cherry (Pleasant Prairie, WI) mini keyboard throughout with the top row of number keys labelled in green from 1 – 9, and the shift and space keys also labelled.

Procedure

The procedure was adapted from one previously described in Trick, et al. (2011).

Pavlovian Training: During initial Pavlovian training four visual stimuli (A, B, C and X; figure 1) were combined into three stimulus pairs, which constituted the trials (AX, BX, CX) and which predicted the occurrence of a reward (study 1)/aversive noise (study 2) with a 90%, 50% or 10% probability, respectively, approximating the design of (Hogarth, Dickinson, et al., 2008). The stimuli were presented in compound with the common stimulus X (Wagner, 1969) in order to assess selective attention for the concurrently presented stimuli A, B and C, which were informative of the trial outcome. The four visual stimuli shown in figure 1 were assigned to the role of A, B, C and X in counterbalanced order across participants.

Each trial started with a fixation cross in the centre of the screen. Once the participant pressed the space bar the fixation cross turned yellow. At this point participants pressed and held the shift key, which terminated the cross and presented a stimulus pair, with the cues 10.5cm either side of the location previously occupied by the fixation cross. The stimulus-pair remained on screen as long as the shift key was held and this represented the **observing time**. Once the shift key was released the stimulus-pair vanished, and the expectancy question "How likely is the 10p/loud noise 1 = unlikely 5 = don't know 9 = likely", was shown in the centre of the screen. Participants answered this question by pressing a green number key between 1 and 9 providing outcome **expectancy ratings** and the question disappeared. Following this, the screen displayed only the grey background for 5 sec and during the last 4 sec of this time the reward outcome ("You gain 10p" was displayed and the participant transferred 10p to their "My Money" tin) or aversive noise (40msec 97dB) could occur at any randomly

selected millisecond. The training phase consisted of 120 trials, arranged in 2 blocks of 60 trials[1].

Mood Induction: The procedure was adapted from one previously described (Robinson, et al., 2009). Participants were presented with 44 (negative or neutral) pictures (Smith, et al., 2005), whilst music was played through Sennheiser PX200 headphones. Participants were instructed to get as deeply as possible into any mood evoked. Firstly, a blank screen was presented and participants were instructed to press the space bar to view the first picture. The picture was displayed in the centre of the screen for 12 seconds, immediately followed by a blank screen, and participants were asked to look at the picture for as long as it was displayed. When the blank screen was displayed again participants pressed the space bar to view the next picture. The music played was *Adagio in G Minor* by Thomas Albinoni for the negative version of the MIP and for the neutral MIP *The Planets, Po. 32: VII. Neptune, the Mystic* by Gustav Holst. Prior to and after the MIP, a set of Visual Analogue Scales (VAS) were administered, to determine self reported mood. Comparison between initial- and post- MIP self reported mood, using VAS, was used to determine the **mood effects of the MIP** and therefore assess the effectiveness of the procedure.

Instrumental training: Participants were then trained to acquire an instrumental response (spacebar pressing) in a procedure identical to Pavlovian training apart from the following modifications. First, holding down the shift key presented two blank grey

[1] Trials within the block were randomised for type (AX, BX, CX) and stimulus location (left, right) Critically, the rewarded outcome occurred in 90% of AX trials (18/20), in 50% of BX trials (10/20) and 10% of CX trials (2/20). Stimulus location was balanced within trials with and without the outcome.

squares in place of the compounds used in Pavlovian training. Participants were instructed that repeatedly pressing the spacebar during the interval following the expectancy question would sometimes lead to the reward/prevent the noise. The reward outcome/noise was scheduled to occur on 25% of trials, and a further 25% were possible by a key press made within the 1-sec window leading up to the scheduled time of the outcome (thus, participant's best strategy was to respond at least once per second across the period following the expectancy question). Consequently, 25% of trials were rewarded (either with the 10p or prevention of the noise) automatically. There were 8 trials of this simple instrumental training. Pavlovian to Instrumental Transfer: The transfer phase followed the design of the instrumental phase except that compounds established in Pavlovian training (AX, BX, CX) were presented randomly, intermixed with blank trials of instrumental training, with equal proportions (16 trials each), over 64 trials. The reward outcome/noise was scheduled for 25% of all trials, with a further 25% possible if instrumental responding was performed effectively (as for instrumental training). Thus the Pavlovian contingencies established in training were not in force in the transfer phase. The number of instrumental responses (space bar presses) made during the variable time window prior to the scheduled time of the reward/noise were recorded to determine the **transfer effect**. This variable time window was matched for trials in which the outcome (reward/noise) was and was not scheduled.

Evaluative conditioning

At the end of the task, the affective evaluation of stimuli was recorded to provide an alternative measure of conditioning. Participants were presented with the individual stimuli A, B, C, and X, in random order, and answered the questions "How anxious does this picture make you?" and "How pleasant do you find this picture?" in random order, on a scale from 1-9 where 1 = not at all, and 9 = extremely. The affective

responses were examined in relation to the impact of cues on attention and instrumental performance.

Statistical analysis

Data were analysed independently for each study and separately for Pavlovian training and transfer sections within each study, and also for aware and unaware participants.

Initial analysis was performed using a 2x3x2 mixed ANOVA with the between factor Condition (2 levels – Negative Mood, Neutral Mood), and within factors Trial (3 levels - AX, BX, CX) and Block (2 levels – block 1, block 2) for the variables Expectancy ratings and Observing times. The Block variable was eventually collapsed because it showed no interesting effects or interactions, to simplify reporting of the key findings. This was followed by post-hoc Bonferroni tests where appropriate, unless otherwise stated. A Greenhouse-Geisser correction was used where required.

Analysis of the PIT effect (number of instrumental responses) was carried out as above with the exception of the factor Trial, which now had four levels (4 levels - AX, BX, CX, Blank) by inclusion of the blank. Other analysis was performed using mixed ANOVA followed by Bonferroni post-hoc testing where appropriate, unless otherwise stated.

Results

Biographical data were collected from both aware and unaware participants and demonstrated no differences between conditions.

Awareness of training contingencies

In the final half of Pavlovian training (60 trials) each participant produced 20 expectancy ratings for each of the three trial types; AX, BX and CX. For each participant the three trial types were compared in the second block of trials using a one-way, within-subjects ANOVA. If there was a significant main effect of trial type, and the direction of effect was veridical with the scheduled Pavlovian contingencies (i.e., AX>BX>CX) the participant was labelled 'aware', otherwise the participant was labelled 'unaware'. The awareness criteria bisected participants who ranged on a continuum of predictive knowledge, rather than participants falling on a step function of predictive knowledge (Lieberman, Sunnucks, & Kirk, 1998). The awareness criteria therefore isolate participants who achieved the greatest predictive knowledge in the training provided. The aware group was analysed independently to confirm the co-occurrence of predictive knowledge and conditioned responding (Hogarth & Duka, 2006 ; Lovibond & Shanks, 2002), data for unaware participants are shown tabulated where appropriate (table 1).

Table 1

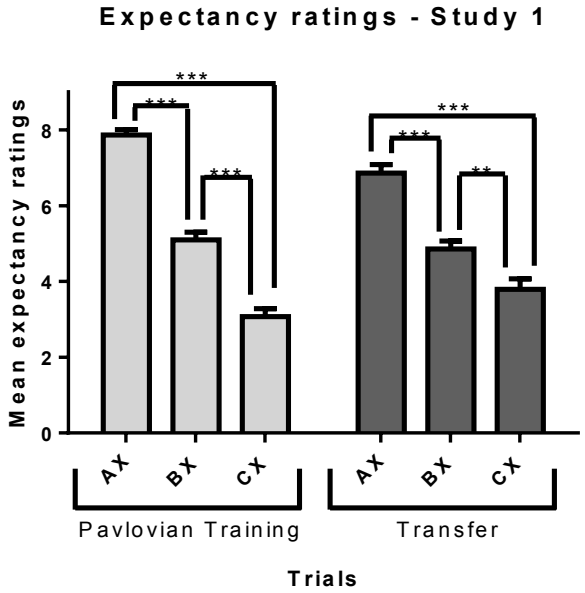
Data from unaware participants

(Conditions collapsed)	**Mean**		**Standard Deviation**	
	Study 1	**Study 2**	**Study 1**	**Study 2**
Pavlovian Training Phase				
Training Expectancy (1-9) - AX	5.21	6.51	0.71	1.00
Training Expectancy (1-9) - BX	5.37	6.33	0.88	0.89
Training Expectancy (1-9) - CX	5.11	4.44	0.54	1.27
Training Observing times (ms) - AX	2605.44	2683.95	908.83	1491.82

Training Observing times (ms) - BX	2969.64	2553.95	1009.79	1317.75
Training Observing times (ms) - CX	3096.74	2525.63	901.04	1226.57
Transfer Phase				
Transfer Expectancy (1-9) - AX	5.23	5.88	1.02	1.70
Transfer Expectancy (1-9) - BX	5.30	6.23	1.16	2.31
Transfer Expectancy (1-9) - CX	5.47	5.38	1.10	1.55
Transfer Observing times (ms) - AX	2084.50	1814.43	1027.43	1292.81
Transfer Observing times (ms) - BX	1561.73	1295.11	635.15	942.50
Transfer Observing times (ms) - CX	2222.11	1738.71	938.37	1062.20
Transfer number of responses - AX	6.97	8.94	7.67	5.97
Transfer number of responses - BX	8.01	10.21	8.35	6.83
Transfer number of responses - CX	7.79	8.45	8.24	5.94
Transfer number of responses - Blank	8.62	12.03	8.24	5.86
Evaluative conditioning				
Anxiety rating (1-9) – A	3.67	4.50	2.58	2.61
Anxiety rating (1-9) – B	3.95	3.50	2.42	2.71
Anxiety rating (1-9) – C	4.38	3.83	2.89	2.59
Anxiety rating (1-9) – Blank	3.52	4.75	2.60	2.67
Pleasantness rating (1-9) – A	5.62	4.75	2.40	2.45
Pleasantness rating (1-9) – B	5.14	5.67	1.74	2.87
Pleasantness rating (1-9) – C	4.95	5.58	2.38	2.75
Pleasantness rating (1-9) - Blank	5.24	3.08	2.68	1.68

Expectancy ratings

Figure 2 shows the expectancy ratings for AX, BX and CX trials during training for aware participants. In both studies aware participants recorded significantly different ratings for each stimulus, study 1: $F(2, 60) = 153.30$, $p < .001$ and study 2: $F(2, 60) = 314.69$, $p < .001$, but the unaware group did not. These effects were determined by the pre-selection of aware and unaware groups. These differential expectancies were maintained into transfer (shown in figure 2), such that in the aware group, there was a significant main effect of trial type; study 1: $F(2, 60) = 44.54$, $p < .001$ and also in study 2: $F(2, 60) = 46.66$, $p < .001$. The unaware group showed no main effect of trial type. These results were consistent for all experimental groups and the data for aware participants were therefore collapsed.



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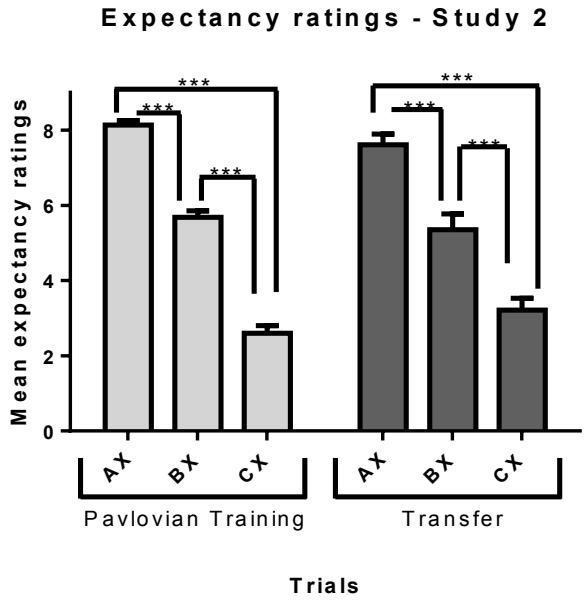
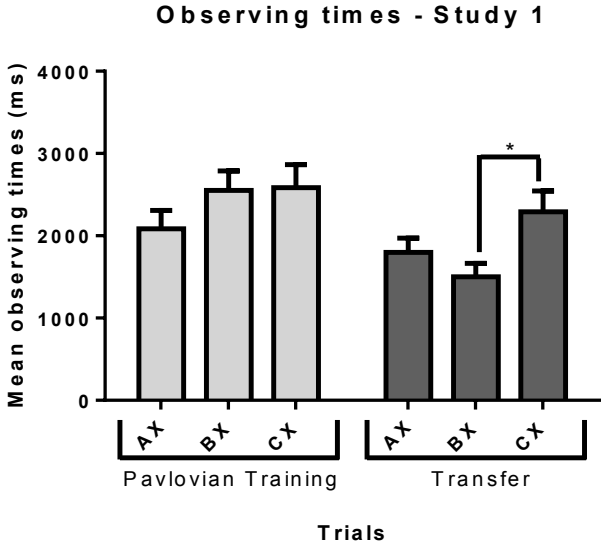


(b)

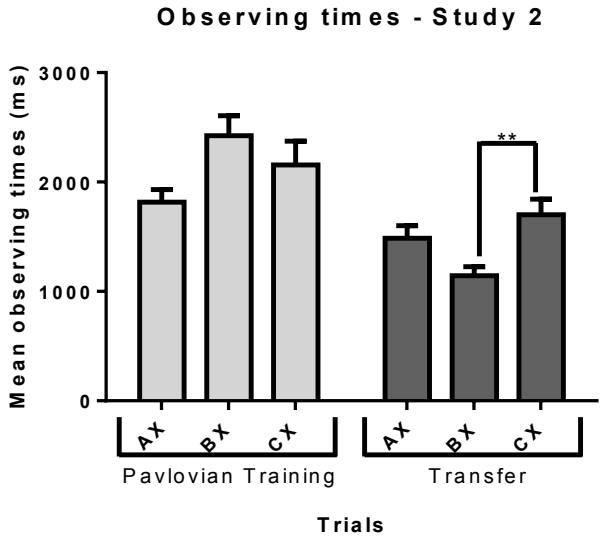
Figure 2: *Mean expectancy ratings following the presentation of trials AX, BX, CX during Pavlovian training and Transfer phase for aware participants for study 1 (a) and study 2 (b). No differences were observed between the conditions and the data were therefore collapsed generating n=32 per bar graphically represented. Error bars represent standard error (SE) of mean. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

Observing time

Figure 3a and 3b show observing times (ms) during Pavlovian training and transfer for study 1 and study 2 respectively. In aware participants, there was a significant main effect of trial type, study 1: $F(2, 60) = 9.39$, $p < .001$; study 2: $F(1.38, 41.41) = 15.72$, $p < .001$, with observing times being overall longest in BX trials compared to AX and CX trials. There was no significant trial effect for unaware participants. By contrast, during transfer, BX observing times were shorter compared to AX and CX and there was a significant main effect of trial type, study 1: $F(1.57, 47.13) = 12.94$, $p < .001$; study 2: $F(1.62, 48.72) = 16.23$, $p < .001$. This result is consistent with the results of (Hogarth, Dickinson, et al., 2008) and further demonstrates that observing time can be used to index the predictive uncertainty of stimuli; this pattern was consistent for all experimental groups and the data for aware participants were therefore collapsed.



(a)



(b)

Figure 3: *Mean observing times (ms) for trials AX, BX, CX during the Pavlovian training and the transfer phase for aware participants for study 1 (a) and study 2 (b). No differences were observed between the conditions and the data were therefore collapsed generating n=32 per bar graphically represented. Error bars represent SE of mean. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.*

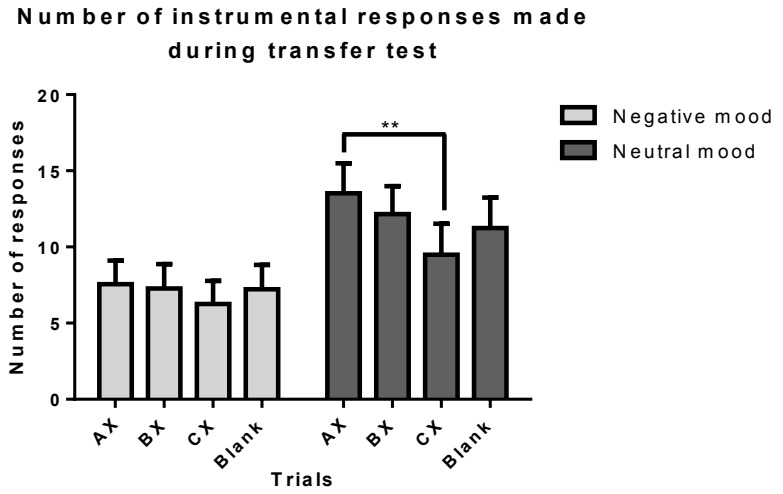
The effects of the MIP on self-reported mood

The change in self-reported mood, calculated by determining the difference in response to VAS before and after the MIP, for aware participants was analysed. There was a significant overall change by condition interaction in study 1 ($F(1, 30) = 30.43$, $p<0.001$), as well as a main effect of mood type (change in happiness/sadness: $F(1, 30) = 56.30$, $p<0.001$). For aware participants who took part in study 2 there was also a significant overall mood change by condition interaction ($F(1, 30) = 8.47$, $p=0.007$), as well as a main effect of mood type (change in happiness/sadness: $F(1, 30) = 47.39$, $p<0.001$). These effects are explained by participants in the negative condition (study 1 – mean: 3.75; SEM: 0.50 & study 2 – mean: 3.81; SEM: 0.52) reporting a greater increase in sadness after the MIP than those in the neutral condition (study 1 – mean: 0.16; SEM: 0.54 & study 2 – mean: 1.64; SEM: 0.70) in both study 1 ($p<0.001$) and study 2 ($p<0.05$); those in study 1 also reported a greater decrease in happiness after negative MIP (study 1 – mean: -3.38; SEM: 0.39 & study 2 – mean: -2.81; SEM: 0.57) than those in the neutral condition (study 1 – mean: -0.93; SEM: 0.32 & study 2 – mean: -1.06; SEM: 0.52; $p<0.001$). It is possible that the effects of the MIP may have been reduced in study 2 as the stimuli used in the PIT procedure predicted an auditory outcome and may have limited the auditory effect of the MIP.

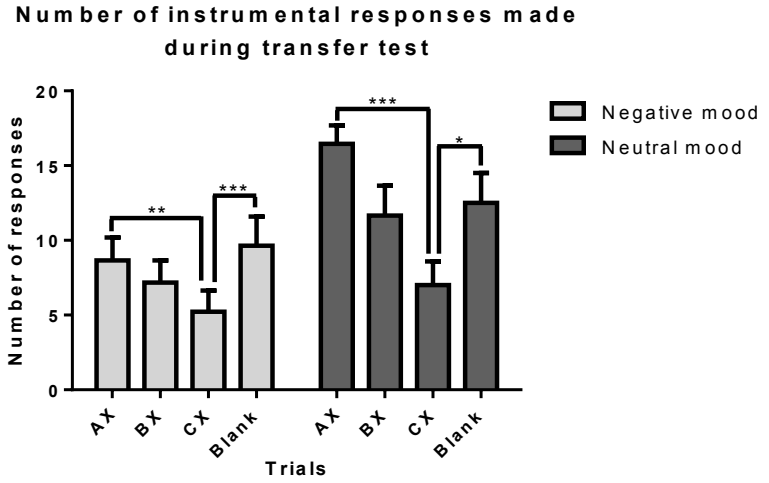
Transfer effect

Figure 4 shows the number of key press responses produced in AX, BX, CX and blank trials for aware participants for each condition. In study 1, there was a significant stimulus effect ($F(1.72, 51.45) = 5.53$, $p = .009$), and also in study 2 ($F(3, 90) = 14.77$, $p < .001$), indicating a PIT effect. Additionally, in study 2 there was also a significant

stimulus by condition interaction ($F(3, 90) = 3.32$, $p = .024$), explained by a higher number of responses being made in the neutral condition.



(a)



(b)

Figure 4: *Mean instrumental responses following presentation of AX, BX, CX and blank trials in study 1 (a) and study 2 (b) during the Transfer phase made by aware participants for each condition (n=16 per condition). Error bars represent SE of mean. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.*

Evaluative conditioning

Table 2 shows the pleasantness and anxiety ratings to stimulus reported at the end of the task for aware participants. Within study 1, there was a main effect of stimuli for both anxiety ($F(3, 90) = 4.28$, $p = .007$) and pleasantness ($F(3, 90) = 4.51$, $p = .005$) for aware participants; which was absent in unaware participants (anxiety: $F(3, 57) = 0.70$, $p = .56$; pleasantness: $F(3, 57) = 0.38$, $p = .768$). For study 2, there was also a main effect of stimulus for anxiety rating for the aware group ($F(3, 90) = 5.43$, $p = .02$), but not in the unaware ($F(3, 30) = 0.57$, $p = .64$). Also within study 2, for pleasantness ratings there was a significant stimulus by condition effect ($F(3, 90) = 3.7$, $p = .015$), but there was not a main effect of stimulus for either the aware ($F(3, 90) = 1.85$, $p = .14$) or unaware ($F(3, 30) = 1.67$, $p = .19$) groups.

Table 2

Mean emotional ratings (SEM) made by aware participants

Measurement	**Study/Condition**	**Stimuli**			
		A	**B**	**C**	**X**
	Study 1				
Anxiety ratings					
	Negative mood condition mean	2.50	3.44	4.00	3.25
		(0.30)	(0.54)	(0.51)	(0.38)
	Neutral mood condition mean	2.63	2.75	3.19	1.75
		(0.56)	(0.40)	(0.55)	(0.25)
Pleasantness ratings					
	Negative mood condition mean	6.38	5.06	4.25	4.75
		(0.58)	(0.53)	(0.48)	(0.45)
	Neutral mood condition mean	6.06	4.88	5.00	6.00
		(0.60)	(0.44)	(0.46)	(0.36)
	Study 2				
Anxiety ratings					
	Negative mood condition mean	5.44	4.63	3.56	5.00
		(0.81)	(0.64)	(0.63)	(0.67)
	Neutral mood condition mean	6.31	4.94	3.56	4.44
		(0.67)	(0.61)	(0.59)	(0.59)
Pleasantness ratings					
	Negative mood condition mean	3.38	4.13	6.69	4.50
		(0.63)	(0.61)	(0.64)	(0.55)
	Neutral mood condition mean	5.25	5.06	4.69	5.44
		(0.71)	(0.60)	(0.76)	(0.35)

Discussion

The current studies investigated the impact of induced negative mood on motivation to perform an instrumental response to gain reward and independently to avoid punishment, and, also links to previous work on reward devaluation. The results of the present studies have shown that successful Pavlovian training generated differential expectations about the probability of the reward/aversive outcome in AX, BX and CX trials. These expectancies were maintained in the transfer phase (post-MIP) despite these contingencies no longer being in force, which is interesting in itself as previous studies have demonstrated a link between serotonin and reduced discrimination between expected gains (Rogers, et al., 2003). In our studies for both aversive and rewarding outcomes the participants who had undergone negative mood induction maintained their expectancies of the outcome as did those in the neutral condition. This may, however, demonstrate dissociation between acute tryptophan depletion and experimentally induced mood (as in the present study), the effects of which are unclear and would be an interesting focus for future study.

Attention to the stimuli (observing times) in the current studies were shown to be linked with uncertainty, according with Pearce & Hall (1980) such that during Pavlovian training, observing times were greatest for BX (the uncertain predictor) as has been previously demonstrated (Hogarth, Dickinson, et al., 2008). In contrast, during transfer, observing times were reversed such that attention to BX was reduced below that for AX and CX in accordance with previous data (Trick, et al., 2011), which hypothesised this effect might be due to prediction error. It is interesting to note that induced mood did not affect observing responses during the transfer stage (post-MIP), indicating dissociation between mood and prediction error in contrast to mood and motivation as reflected by the number of instrumental responses performed.

The number of instrumental responses for each set of compound stimuli in the neutral condition increased linearly in accordance with the expectancies/probabilities learned in Pavlovian training (AX>BX>CX), thus demonstrating this transfer effect is related to explicit prediction of the outcome (e.g., Hogarth, et al., 2007; Trick, et al., 2011, etc). This was the case for both aversive and appetitive models (study 2 and study 1 respectively). This dissociation between attention (observing times being driven by uncertainty) and behaviour (instrumental responding driven by prediction) is interesting with regard to the present study as observing responses were not affected by the manipulations (MIP); however, the transfer effect was altered in the negative mood conditions in both paradigms.

Data in the present studies also demonstrate successful manipulation of mood, such that the negative MIP resulted in greater self reported feeling of sadness in both studies, accompanied by a reduced feeling of happiness in study 1 when compared to those in the neutral condition. Additionally, expectancy ratings were maintained by the aware participants into transfer (but with no difference between the two mood groups), prior to which the MIP was completed, indicating that the MIP had no effect on contingency knowledge and therefore supports the suggestion that the reduction in transfer effect in the negative mood condition was driven by a reduced motivation to perform the response despite maintaining associated knowledge of the outcome. This is in accordance with previous studies, such that those in a negative state may become more accurate, but slower at responding (Cools, et al., 2005). In Cools, et al., (2005) the effects of acute tryptophan depletion on a reaction time task were tested, and those participants who had undergone the serotonin manipulation reduced the speed of their responses but increased the accuracy. Volunteers in the control condition demonstrated increased speed and lower accuracy on trials predictive of high reinforcement certainty

when compared with trials for cues associated with lower reinforcement certainty; this coupling was also influenced in the manipulation condition. As discussed the link between serotonin and motivation is already well established (Cools, et al., 2005), as is the link between serotonin and negative mood. The present work adds to our knowledge regarding the processes by which mood effects learning and behavioural processes as studies previously have demonstrated a link between serotonin and sensitivity to reward reinforcements, in particular punishment (Blair, et al., 2008; Cools, Robinson, et al., 2008).

An important caveat should be noted when comparing the current results to previous data. The present studies utilised experimentally induced mood achieved via a musical and visual mood induction procedure designed to alter state mood, as opposed to other studies, which have used acute tryptophan depletion alone or in combination with a mood induction procedure. Interestingly, the latter of which would most likely provide the most robust results as mood state has been shown to influence the effects of acute tryptophan depletion (Robinson, et al., 2009). Irrespective of the mood state procedure used, previous findings are similar to the ones presented here (Cools, et al., 2005; Cools, Robinson, et al., 2008) that a reduction in response was observed in the negative mood condition, but accuracy maintained (as observed with expectancy in the present studies). In addition, the present results are in accordance with previous reports (Dayan & Huys, 2008; Rogers, et al., 2003) such that negative mood does not affect discrimination between expected outcomes when they are aversive and may drive a reduction in motivation to seek reward/avoid punishment by altering the way in which reward cues are processed. An enhancement in punishment prediction following acute tryptophan depletion has also been demonstrated previously, but no effect on reward prediction (Cools, Robinson, et al., 2008). This dissociation is interesting with regard to the current

data as in study 1, where a reward outcome was utilised, the number of instrumental responses performed for each set of stimuli to gain the reward was reduced and the PIT effect (responses AX>BX>CX) was abolished. However, in study 2 when the stimuli were predictive of an aversive outcome the response to avoid this outcome was reduced but the PIT effect was maintained. This is also in agreement with certain theories that hypothesise serotonin (reduced under tryptophan depletion procedures) is involved in punishment prediction, but not reward (Daw, et al., 2002). Negative mood has been extensively linked to serotonin (Cools, et al., 2005) and negative mood induction has also been shown to increase salivary cortisol (Brown, Sirota, Niaura, & Engebretson, 1993). The link between serotonin and motivation and impulsivity is well established (Cools, et al., 2005) such that a reduction in serotonin is linked to a reduction in motivated actions, and can lead to a reduction in reward seeking behaviour. It is therefore possible that state negative mood is linked to a decrease in motivation more generally and thus motivation is reduced when the outcome is to avoid punishment and also to gain reward in the natural environment, but is dissociated from the expectancy knowledge of that same reward, and also prediction error. Our results indicate that the observed reduction in motivation is driven by an attenuated sensitivity to the reward itself, such that the reward is devalued, and not a reduction of the impact of the conditioned signals as expectancy was maintained (Rogers, et al., 2003). The reduction in motivation to gain reward demonstrated in the present study may provide an insight in to treating drug addiction and is also relevant for the link between depression and cognitive impairment. The data may also demonstrate a possible link between current mood state and reward value. The present findings, although in a non-clinical sample, indicate this is likely.

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Chapter 4

Paper 2: Pavlovian-instrumental transfer in humans is sensitive to changes in probability and current value of reward

Abstract

Pavlovian conditioning endows reward-associated stimuli with the ability to modulate goal-directed actions for that same reward (Pavlovian-Instrumental transfer; PIT). The present study examined in humans the extent to which PIT is affected by changes in the value of the outcome. Participants initially learned to discriminate three visual stimuli, A, B and C, predicting the occurrence of monetary reward (10 pence) with 90%, 50% or 10% probability, respectively. Next, the value of the outcome was either devalued (by means of a separate High-win or Low-win task) or remained unchanged. Following a phase of instrumental training, in which responding yielded 10p with a 50% probability on a VI schedule of reinforcement, a transfer test was conducted during which the Pavlovian cues were presented non-contingent on instrumental responding. The change of reward value affected the rigor of instrumental performance and abolished the PIT effect. However, the same changes in outcome value did not affect the expectancy of or emotional reactivity to the cues. These data suggest that in Pavlovian learning, apart from the nature of outcomes, the value of outcomes is encoded such that changes in outcome value prevent transfer of a Pavlovian cue's incentive properties to alter goal-directed action.

Key words: learning, human, devaluation, addiction, attention

Introduction

Like non-human animals, humans readily acquire simple Pavlovian stimulus-outcome (S-O) associations when neutral stimuli in the environment are repeatedly paired with biologically significant outcomes (e.g., rewards) in the laboratory (Everitt, et al., 2003; Everitt & Robbins, 2005; Hogarth & Chase, 2012). Similarly, human subjects will acquire simple instrumental action-outcome (A-O) associations when outcomes are presented contingent on performance of a simple (operant) response (Dickinson, 2001). Though typically studied separately, and psychologically and neurobiologically dissociable (Everitt, et al., 2008; Everitt, et al., 2003; Everitt, et al., 2001; Everitt, et al., 2007; Everitt & Robbins, 2005), it is now well established that S-O and A-O associations may interact to control motivated behaviour such that Pavlovian cues can modulate instrumental or goal-directed action (Hogarth, et al., 2005). For instance, in Pavlovian-Instrumental Transfer (PIT) nominal reward-associated cues can be shown to maintain and potentiate on-going instrumental performance when presented non-response-contingently and in the absence of primary reward.

Since Estes' (Gutman & Estes, 1949) first demonstration of PIT, the phenomenon has been reproduced in several animal species, including rats (e.g. Colwill & Rescorla, 1990; Lovibond, 1983, etc), mice (O'Connor, Stephens, & Crombag, 2010) and more recently in humans (Bray, et al., 2008; Hogarth, et al., 2007; Talmi, et al., 2008). An important focus of these and other investigations has been identifying the underlying psychological and neurobiological mechanisms, as well as understanding the conditions and variables that determine the magnitude and nature of PIT. For example, we (Trick, et al., 2011) recently reported, using a variation of an outcome-selective PIT procedure, in which the probabilities of an aversive outcome delivery were varied, that the magnitude of transfer is closely linked to outcome prediction (90% > 50% > 10%).

Interestingly, this “negative” transfer effect on performance was dissociable from attentional bias to the Pavlovian conditioned cues.

Apart from being a useful procedure to study the basic psychological and neurobiological mechanisms by which S-O and A-O based learning interact in the laboratory, many “day-to-day” motivated actions are regulated through PIT-like mechanisms. Moreover, PIT is thought to play a role in promoting maladaptive behaviours including excessive overeating (Galarce, Crombag, & Holland, 2007) or addiction to drugs of abuse (Berridge, Robinson, & Aldridge, 2009; Everitt, et al., 2001). Thus, environmental cues associated with drug taking (e.g. drug paraphernalia, location of consumption etc.) may maintain and promote compulsive drug-seeking and trigger relapse through PIT-like processes (Berridge, et al., 2009; Crombag, Bossert, Koya, & Shaham, 2008; Everitt, et al., 2001; Glautier, et al., 1994).

Nonetheless, the precise mechanisms by which Pavlovian and instrumental learning processes interact to promote motivated action in general, or drug seeking and taking in particular, are very much a subject of debate. One hypothesis states that cue-elicited drug seeking is a manifestation of goal-directed action, driven by a mental representation of the contingency between response and outcome (Holland, 2007; Olmstead, et al., 2001). Alternatively, Tiffany (1990) proposes that addict’s drug taking reflects a shift of control from instrumental A-O to habitual stimulus-response (S-R) associations (Tiffany, 1990; Tiffany & Drobes, 1990), whereby, once the S-R association has been established, the conditioned cue alone is capable of eliciting a (conditioned) drug seeking response, in the absence of conscious retrieval of any outcome expectancies.

A ‘litmus test’ for the existence for such S-R habits is the ‘resistance to devaluation’ procedure to demonstrate that changes in outcome value (e.g., by pairing the outcome with experimentally-induced, e.g., systemic lithium chloride injections, malaise) are not reflected in changes in performance. Conversely, if performance is goal-directed it would be expected to decrease or discontinue following outcome devaluation, presumably because the reduction in goal-value would render it inadequate to motivate behaviour.

From the animal literature it becomes apparent that transfer effects are typically resistant to devaluation (e.g. Holland, 2004; Rescorla, 1994, etc) and thus that PIT performance is not mediated by the expected incentive value of the outcome. Thus, Rescorla (1994) notes that Pavlovian cue-activated representations incorporate the sensory aspects of the outcome (S-O associations) learned during Pavlovian training to activate the response (O-R associations) irrespective of the current value of the outcome.

On the other hand, recent findings from studies on PIT in humans are less consistent. Whereas Allman et al, (2010) showed that outcome specific transfer can be modulated by altering the current value of the reinforcer, other studies (Hogarth, 2012; Hogarth & Chase, 2011) showed no effect of devaluation on performance. One obvious account for these differences is that these studies differed in the way they ran the PIT and devaluation procedure. Allman, et al. (2010) used a scenario-based procedure in which participants, during the Pavlovian training phase, learned that companies (presented by their logos) were using either Hong Kong or US dollars as their currencies, after which they were informed that one of the currencies used had become worthless (devalued). Hogarth 2012 (Hogarth, 2012; Hogarth & Chase, 2011), on the other hand, used as conditioned stimuli pictorial representations of the reinforcers (cigarette or chocolate),

to which participants had (presumably) been exposed extensively prior to the experiment, and used selective satiation to devalue the reinforcers.

The present experiment used an implicit (between task) reward devaluation procedure and a probability-based Pavlovian conditioning procedure [similar to the aversive one used by us previously in Trick, et al., (2011)] to examine further the conditions under which current reinforcer value can modify PIT. In this procedure Pavlovian cues predict reward with different degrees of uncertainty (90%, 50% or 10%) and develop the ability to activate performance in accordance with the probability or level of uncertainty by which the reinforcer previously occurred. Introduction of such predictive uncertainty during Pavlovian conditioning may be a critical determinant of the qualitative nature, the underlying neurobiological mechanisms, and the behavioural consequences of associative learning (Anselme, Robinson, & Berridge, 2013; Davey & Cleland, 1982; Fiorillo, et al., 2003; Linnet et al., 2012) and, we predict, of the sensitivity of performance to changes in value of the reward.

Materials and Methods

Participants

Seventy-eight healthy subjects (40 females), who were verified as not taking medication using a medical health questionnaire, were recruited. Ethical approval was obtained from The University of Sussex ethics committee, and the study run in accordance with the Declaration of Helsinki. All subjects were required to give written informed consent prior to participation and were informed that they could withdraw at any time. At the end of the experiment the subjects were fully debriefed and compensated for their time with £15 in addition to their earnings in the experiment. Subjects were asked to attend

the laboratory on a single occasion and instructed to abstain from alcohol for twelve hours, and from anything high in caffeine immediately before, the test session.

Tasks

The tasks were presented on a 20" Dell P1130 monitor (Dell Inc, Berkshire, UK) and programmed using E-prime v1.1 software (Psychology Software Tools Inc.; Pittsburgh, PA). The 4 visual stimuli used (see figure 1) were black patterns displayed on a grey background, 10.2cm squared at a resolution of 1280 x 1024. Text font and size were Times New Roman 25pt. Participant responses were collected via a Cherry mini keyboard (Pleasant Prairie, WI) with the shift and space keys labelled in green, and the top row of numeric keys labelled (1-9) also in green.

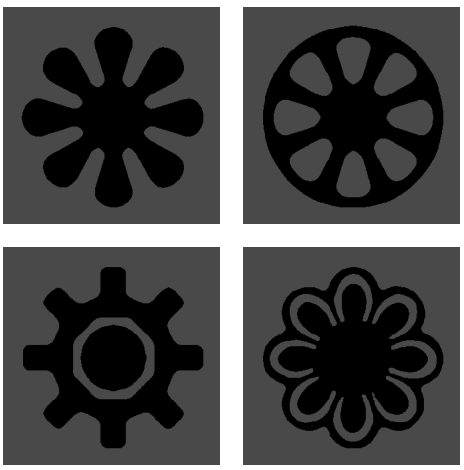


Figure 1: *Materials used during the computerised task throughout the PIT procedure; the four visual stimuli assigned to A, B, C and X in a counterbalanced fashion*

Procedure

The procedure was adapted from one previously described (Trick, et al., 2011) and consisted of the following phases:

Pavlovian discrimination training phase: During the initial Pavlovian training phase participants received repeated trials during which 1 of 3 different visual compound cues were presented (AX, BX or CX) that predicted the occurrence of a reward (gain of 10 pence) with a 90%, 50% or 10% probability, respectively. Thus, like (Hogarth, Dickinson, Austin, Brown, & Duka, 2008) the target cues informing the trial outcome were presented in compound with a common stimulus X (Wagner, 1969). Attention for the concurrently presented cues A, B and C was assessed by measuring the time that participants spent observing the stimuli (see below). The 4 images that made up stimuli A, B, C and X were counterbalanced across participants.

Each trial started with a fixation cross appearing in the centre of the screen. Once the participant pressed the space bar the fixation cross turned yellow. At this point participants pressed and held the shift key, which removed the cross and presented 1 of the 3 stimulus pairs, with the individual images located 10.5cm on either side of the fixation cross location. The stimulus-pair remained on screen for as long as the shift key was pressed, thereby providing a measure of observation time. Once the shift key was released and the stimulus-pair vanished, the expectancy question “How likely is the win of 10p: 1 = unlikely 5 = don't know 9 = likely”, was displayed in the centre of the screen. Participants answered this question by pressing a green number key between 1 and 9, providing outcome expectancy ratings. Following the outcome expectancy response, the screen display turned grey for 5 sec and during the last 4 sec of this period the reward outcome was presented. On trials where the reward outcomes was “You

gain 10p", participants transferred 10p to their "My Money" tin. The Pavlovian training phase consisted of 120 trials, arranged in 2 blocks of 60 trials and the trials within each block were randomised for type (AX, BX, CX) and stimulus location (left, right of fixation cross location), and stimulus location was balanced across trials with and without the outcome. Critically, the rewarding outcome occurred in 90% of AX trials (18/20), in 50% of BX trials (10/20) and 10% of CX trials (2/20).

Outcome revaluation phase: Following Pavlovian S-O training, participants underwent an outcome-revaluation manipulation akin to the selective satiation procedures used in non-human subject devaluation studies (Holland, 2004; Rescorla, 1994). To this end, participants assigned to the revaluation condition received the following instructions:

"You will now take a break from the task and play a card game. Each round will begin with the presentation of 5 cards. You must choose one of these cards by pressing a green number key 1 - 5. Every time you pick a black card you will win £1. You will get 14 chances to choose a card. At the end you will be told how much you have won in total. Press the space bar to begin."

Participants were next presented with five cards placed face-down and selected each card using a keyboard number key. Participants were pre-determined to win either £2 (low win) or £12 (high-win) and were given a bag of fourteen £1 coins to transfer the winnings to their "My Money" tin.

Participants assigned to the control or (maintained) condition received the following instructions*:*

"You will now take a break from the task and play a card game. Each round will begin with the presentation of 5 cards. You must choose one of these cards by pressing a green number key 1 - 5. You will get 14 chances to choose a card. See how many black

(counterbalanced with red) *cards you pick. Press the space bar to begin."* All participants were pre-determined to select seven black cards and seven red.

Instrumental training: Participants were next trained to acquire an instrumental response (pressing the keyboard spacebar) during 12 trials of training. To this end, participants were asked on each trial to press and hold down the shift key in order to be presented with two blank grey squares and to release the shift key in order to give their outcome expectancy ratings. Participants were instructed that repeatedly pressing the spacebar during the interval following the expectancy question would sometimes lead to a reward (earning 10p). The reward outcome was scheduled to occur automatically on 25% of trials, irrespective of the subject's response. A further 25% of rewarded trials were possible by making a key press during the variable 1-sec window leading up to the scheduled time of the outcome (this one second window occurred at a variable time during the interval following the expectancy question and the scheduled time of the outcome). Consequently, the maximum rewarded trials could be 50% of all trials (25% were always rewarded).

Pavlovian-instrumental transfer: The transfer test followed the same design as the instrumental training phase except that the compound cues established during Pavlovian discrimination training (AX, BX, CX) were presented randomly intermixed with the blank grey square trials, with equal proportions (16 trials each), over 64 trials. The reward outcome was scheduled for 25% of all trials, with a further 25% possible if instrumental responding was performed effectively. The outcome contingencies available during Pavlovian training were not available during the test for transfer. The number of instrumental responses (space bar presses) made during the time window was recorded and the window was matched for trials in which the reward was and was not scheduled.

Evaluative conditioning

At the end of the transfer test, the affective evaluation of the Pavlovian cues was recorded to provide an alternative assay of conditioning. Participants were presented with the individual stimuli A, B, C, or X, in random order, and answered the questions "How anxious does this picture make you?" and "How pleasant do you find this picture?". Evaluative responses were again recorded on a scale from 1-9 where 1 = not at all and 9 = extremely.

Statistical analysis

Pavlovian training: Data from the Pavlovian training and transfer phases were analysed separately and results from the 'aware' and 'unaware' participants were also analysed separately. Expectancy ratings and observation times were analysed using mixed ANOVAs with the between-factor revaluation Condition (3 levels; high-win, low-win, control) and within-factors Trial (3 level; AX, BX, CX) and Block (2 levels; block 1, block 2). Where appropriate, this was followed by Bonferroni-corrected post-hoc tests unless noted otherwise. A Greenhouse-Geisser correction was used where required.

Transfer phase: The Pavlovian transfer effects on instrumental performance were analysed as above except that the factor Trial had four levels, namely AX, BX, CX, and Blank. Additionally, to simplify reporting of the key findings and because no interesting effects or interactions were evident, the Block variable was excluded from the analysis where appropriate.

Results

Awareness of contingencies; aware and unaware group

As in our previous studies, a number of participants (30 out of 78) failed to successfully acquire the Pavlovian (stimulus-outcome) associations. Thus, each participant's expectancy ratings as a function of trial type (AX, BX and CX) during Pavlovian training trials (2^{nd} block, 20 ratings/trial type) were analysed using one-way, within-subjects ANOVAs. A participant was designated as 'aware' if there was a significant effect of trial type, and the direction of his/her expectancy ratings was veridical with the programmed Pavlovian contingencies (i.e., AX>BX>CX). The awareness criteria bisected participants who ranged on a continuum of predictive knowledge, rather than participants falling on a step function of predictive knowledge (Lieberman, Sunnucks, & Kirk, 1998). The criteria therefore isolated participants who achieved the greatest predictive knowledge as a function of Pavlovian training. The 48 participants, determined to be "aware", were assigned to the 'high win' (n = 16; 8 females), 'low win' (n = 16; 8 females) or 'control' (n = 16; 9 females) conditions. Biographical data collected from 'aware' and 'unaware' participants in the 3 experimental conditions are shown in table 1; no significant differences were found.

Table 1

Characteristics of the high win, low win and control conditions (BIS; Barratt Impulsivity Scale)

Characteristic	**High win**	**Low win**	**Control**
	Aware	*Aware*	*Aware*
Sex ratio	M=8;F=8	M=8;F=8	M=7;F=9
Years of age (mean)	22.1 (SD=2.4)	21.8 (SD=3.7)	22.4 (SD=3.0)
Cigarettes smoked per day (mean)	2.4 (SD=5.5)	2.2 (SD=4.1)	1.3 (SD=3.9)
BDI (mean)	2.8 (SD=3.5)	6.1 (SD=8.5)	3.4 (SD=3.2)
BIS factor 1 (mean)	15.9 (SD=3.2)	18.1 (SD=5.4)	15.9 (SD=3.0)
BIS factor 2 (mean)	23.5 (SD=4.1)	23.1 (SD=5.0)	23.7 (SD=3.8)
BIS factor 3 (mean)	23.5 (SD=4.8)	23.8 (SD=4.9)	22.1 (SD=3.6)
	Unaware	*Unaware*	*Unaware*
Sex ratio	M=4;F=1	M=7;F=6	M=4;F=8
Years of age (mean)	21.8 (SD=2.4)	22.9 (SD=3.0)	21.8 (SD=2.9)
Cigarettes smoked per day (mean)	0 (SD=0)	.4 (SD=.7)	2.2 (SD=3.2)
BDI (mean)	3.2 (SD=3.1)	6.1 (SD=4.8)	9.3 (SD=11.0)
BIS factor 1 (mean)	15.2 (SD=2.4)	18.6 (SD=4.4)	17.7 (SD=3.7)
BIS factor 2 (mean)	23 (SD=4.1)	27.0 (SD=5.5)	23.3 (SD=4.6)
BIS factor 3 (mean)	26.8 (SD=1.5)	26.1 (SD=4.7)	24.3 (SD=4.0)
Total number of participants	**21 (16 Aware)**	**29 (16 Aware)**	**28 (16 Aware)**

The data from the 'aware' group were next analysed to confirm the co-occurrence of predictive knowledge and conditioned responding during transfer (Hogarth & Duka, 2006 ; Lovibond & Shanks, 2002). Data from 'unaware' participants (n=30) are shown tabulated in table 2.

Table 2

Data from unaware participants (n=30; 15 females; conditions collapsed)

	Mean	**Standard deviation**
Pavlovian Training Phase		
Training Expectancy (1-9) – AX	5.18	1.61
Training Expectancy (1-9) – BX	5.73	1.34
Training Expectancy (1-9) – CX	5.31	1.28
Training Observing times (ms) – AX	1907.68	1001.07
Training Observing times (ms) – BX	1869.56	971.29
Training Observing times (ms) – CX	1926.92	995.33
Transfer Phase		
Transfer Expectancy (1-9) – AX	5.40	1.73
Transfer Expectancy (1-9) – BX	5.91	1.62
Transfer Expectancy (1-9) – CX	5.49	1.81
Transfer Observing times (ms) – AX	1368.84	807.90

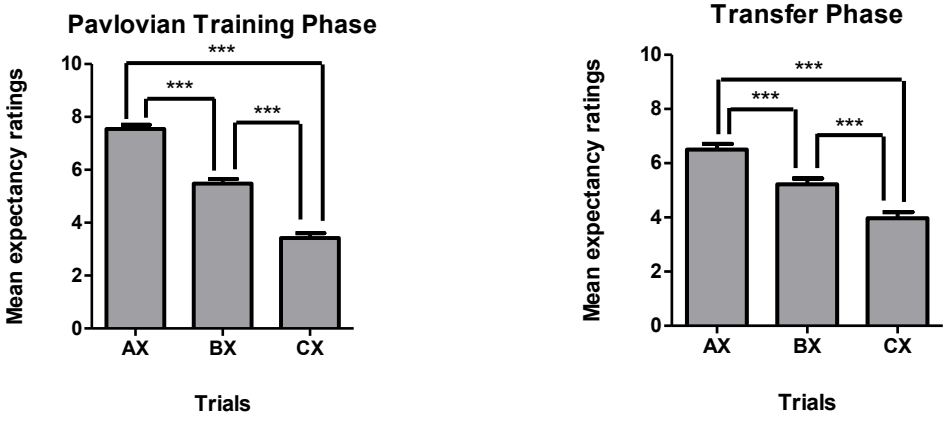
Transfer Observing times (ms) – BX	1104.88	651.32
Transfer Observing times (ms) – CX	1480.30	885.69
Transfer number of responses – AX	10.19	8.21
Transfer number of responses – BX	10.29	8.04
Transfer number of responses – CX	10.18	7.75
Transfer number of responses – Blank	11.20	8.09
Evaluative conditioning		
Anxiety rating (1-9) – A	2.33	1.63
Anxiety rating (1-9) – B	3.00	2.17
Anxiety rating (1-9) – C	3.33	2.34
Anxiety rating (1-9) – X	2.60	1.83
Pleasantness rating (1-9) – A	5.80	2.47
Pleasantness rating (1-9) – B	5.87	1.98
Pleasantness rating (1-9) – C	4.17	2.55
Pleasantness rating (1-9) – X	5.50	2.53

Expectancy ratings

Training phase: As expected, for 'aware' participants, the expectancy ratings for the monetary outcome (10p) were higher for AX trials compared to BX or CX trials, and the lowest expectancy ratings were given for the CX trials (main effect of trial type, $F(2, 94) = 147.91$, partial $\eta^2 = .759$, $p < .001$; figure 2a). Unaware participants did not

show differential expectancy ratings across trials (F(1.43, 41.54) = 2.29, partial η^2 = .073, p = .073 (table 2).

Transfer phase: The aware group's differential expectancies were maintained into the transfer test (a significant main effect of trial type, F(1.73, 81.21) = 32.09, partial η^2 = .406, p < .001; figure 2b). The unaware group showed no main effect of trial type, F(1.43, 41.44) = 1.39, partial η^2 = .046, p = .256. There was no effect of revaluation condition on expectancy ratings of aware or unaware subjects during the transfer test.



(a) **(b)**

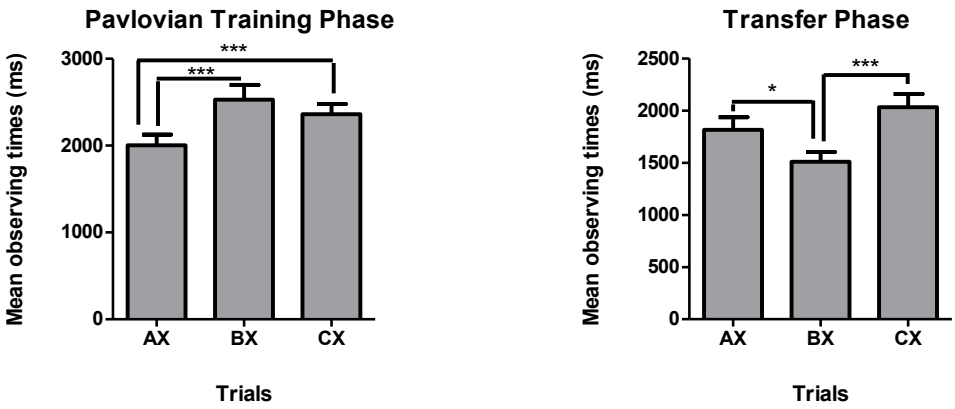
Figure 2: *Mean expectancy ratings following the presentation of trials AX, BX, CX during Pavlovian training (a) and Transfer phase (b) for aware participants. No differences were observed between the conditions and the data were therefore collapsed generating n=48 per bar graphically represented. Error bars represent standard error (SE) of mean. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

Observing time

Training phase: In aware participants, observing time was longer for BX trials relative to the AX trials as well as for the CX trials compared to AX trials (main effect of trial

type, $F(1.64, 76.91) = 19.77$, partial $\eta^2 = .296$, $p < .001$; figure 3a). This result replicated previous findings by Hogarth et al (2008) and further demonstrates that observing time is a sensitive index of the uncertainty of outcome-predictive Pavlovian cues. There was no significant trial effect for unaware participants, $F(2, 58) = .600$, partial $\eta^2 = .020$, $p = .552$.

Transfer phase: By contrast, during transfer, BX observing times were shorter compared to AX and CX for both 'aware' and 'unaware' participants (effects of trial type, $F(2, 94) = 17.99$, partial $\eta^2 = .277$, $p < .001$ and $F(1.60, 46.31) = 8.46$, partial $\eta^2 = .226$, $p = .002$, respectively; figure 3b and table 2). There were no significant effects of revaluation condition on observing time during the test for transfer for aware or unaware subjects.



(a) **(b)**

Figure 3: *Mean observing times (ms) for trials AX, BX, CX during the Pavlovian training (a) and the transfer phase (b) for aware participants. No differences were observed between the conditions and the data were therefore collapsed generating n=48 per bar graphically represented. Error bars represent SE of mean. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

Instrumental performance

Transfer phase: The group which had experienced high-wins during the revaluation task, showed low number of responses (significant main effect of condition $F(2, 45) = 9.88$, $p < .001$; figure 4). A marginal PIT effect was also found (main effect of trial type ($F(2.60, 116.78) = 2.72$, $p = .055$). Importantly, an interaction between trial type and condition which approached significance ($F(6, 135) = 2.08$, $p = .05$) was explained by a higher number of responses in the presence of AX trials compared to CX trials in the control group but not in either of the revalued groups (figure 4). No significant trial effect was found for the unaware participants, $F(2.47, 5.49) = 0.394$, $p = .719$; see table 2.

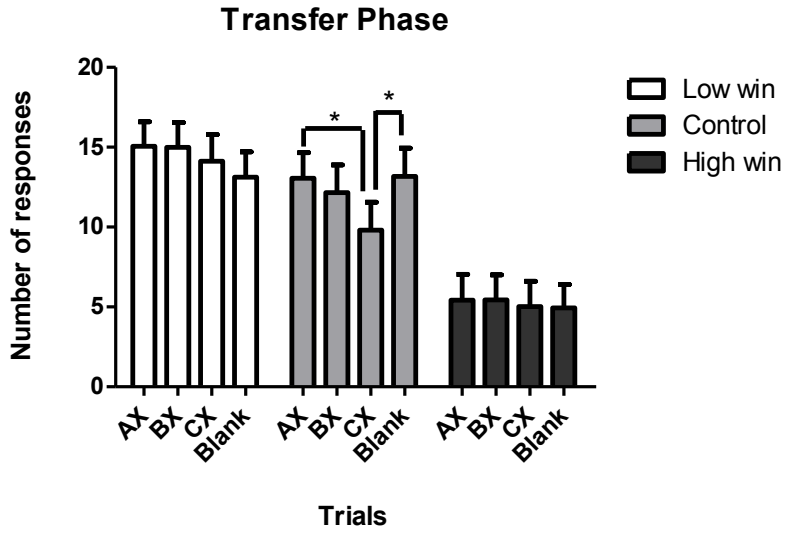
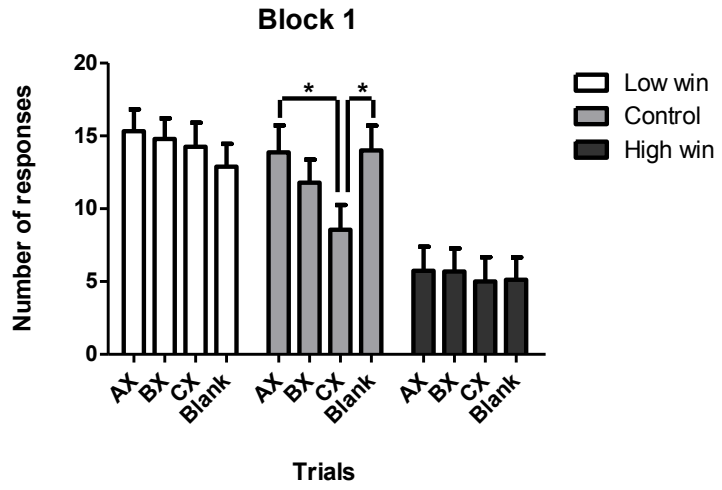
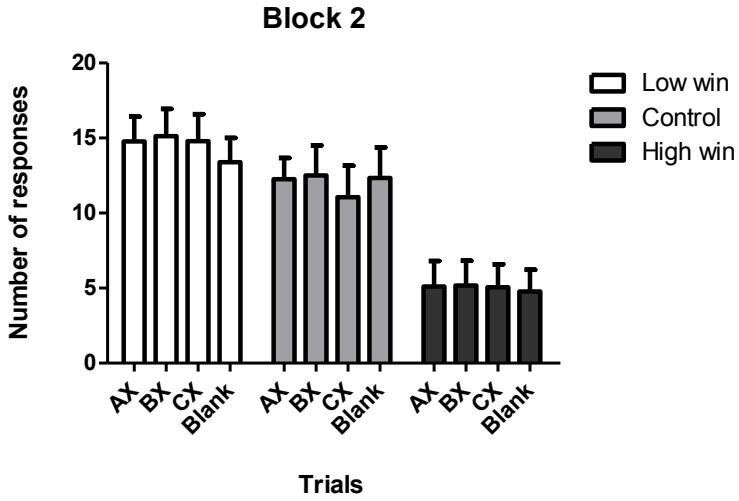


Figure 4: *Mean instrumental responses following presentation of trials AX, BX, CX and blank trials during the Transfer phase for aware participants for each condition (n=16 per condition). Error bars represent SE of mean. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

We further explored the transfer effect for each condition (stimulus by condition interaction) for trial blocks 1 and 2 separately, as previous studies in our lab (unpublished data) typically show small performance decrements (extinction) as (transfer) test sessions progress. A significant stimulus by condition interaction effect was found for block 1 ($F(4.47, 100.50) = 4.03$, $p = <0.01$), explained by a transfer effect by participants in the control condition but not the conditions in which the outcomes were revalued (figures 5a and b). By contrast, and as expected, there was no significant interaction between stimulus and condition during block 2 ($F(4.88, 107.45) = 0.518$, $p = .760$).



(a)

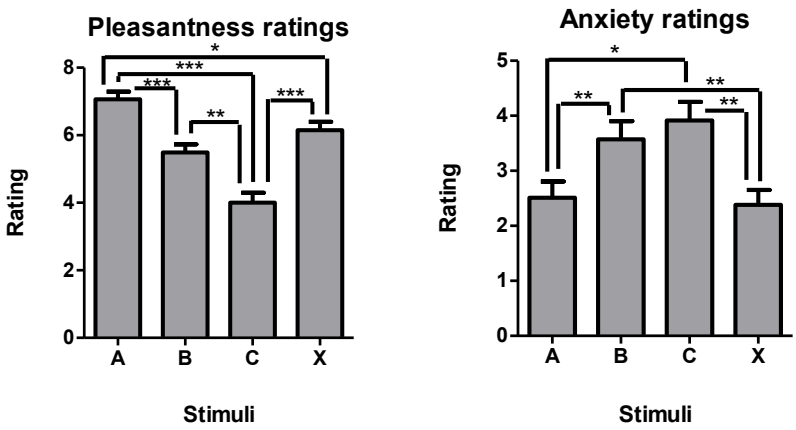


(b)

Figure 5: *Mean instrumental responses following presentation of trials AX, BX, CX and blank trials during the Transfer phase for aware participants for each condition (n=16 per condition), and separately for block 1 (a) and block 2 (b). Error bars represent SE of mean. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

Emotional conditioning

Analysis of emotional ratings by 'aware' subjects (figures 6 a and b) taken immediately following the test for transfer indicated that these subjects rated stimulus A (90% predictability) as significantly more pleasant than all other stimuli, including the "ubiquitous" stimulus X (figure 6a). Thus, a main effect of stimulus ($F(2.57, 118.26) = 26.26$, $p < .001$) was followed by significant ($p < 0.05$) post-hoc comparisons. Additionally, stimulus C (10% predictability) was rated as less pleasant than either stimulus B (50% predictability) or X. Interestingly, also unaware subjects showed a preference for stimulus A as supported by a significant effect of stimulus ($F(3, 87) = 4.24$, $p = .009$) and post-hoc analysis (table 2).



(a) **(b)**

Figure 6: *Mean emotional ratings for each stimulus - A, B, C and for the common stimulus X in the compound. Error bars represent SE of mean. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

Inspection and analysis of the anxiety ratings by aware subjects (figure 6b) revealed almost the mirror image of the pleasantness ratings. Thus, a significant effects of stimulus ($F(2.34, 106.98) = 9.86$, $p < .001$) indicated that anxiety ratings were inversely related to the level of outcome predictability such that stimulus A yielded significantly lower anxiety ratings than stimuli B or C; but no difference was seen between stimulus A and X. Finally, there was no effect of stimulus on ratings of anxiety for unaware participants ($F(3, 87) = 2.01$, $p = .119$; table 2). Finally, no significant effects of revaluation condition were seen on pleasantness or anxiety ratings (data not shown).

Discussion

The present study explored whether simple Pavlovian (S-O) conditioning and transfer of a Pavlovian cue's motivational effects on instrumental (A-O-mediated) performance are sensitive to variations in the level of uncertainly of the Pavlovian association and/or the value of the outcome at the time of testing.

Pavlovian conditioning

As reported by us elsewhere (Austin & Duka, 2010, 2012), the present results show that successful Pavlovian training generated differential expectations about the probability of the reward outcome in AX, BX and CX trials. Specifically, both during the Pavlovian training phase and the transfer test (when these contingencies were no longer reinforced), decreases in the predictability of the rewarding outcome from 90%, to 50% and 10% produced a greater decline in expectancy rating in subjects deemed 'aware' of the contingencies. Moreover, as anticipated by e.g., Pearce and Hall's model of associative learning (Pearce & Hall, 1980), attention to the cues (observing times) was linked with the level of uncertainty, such that during Pavlovian training, observing

times were greatest during BX stimulus trials (the 50% uncertain predictor) compared to stimulus trials that were highly predictive of reward (90%) or non-reward (10%) (Hogarth, Dickinson, et al., 2008).

However, different from the expectancy ratings that show the same pattern during conditioning and transfer, observing times were reversed (relative to training) during the transfer test such that attention to BX was reduced below the levels during AX and CX trials. These results in fact agree with our previous data (Trick, et al., 2011), suggesting that the increase in observing time during BX trials might be related to prediction error. That is, while during the AX and CX trials on the test for transfer the contingencies changed from 90% or 10%, to 50%, respectively, they remained the same during BX trials when training and test contingencies remained at 50%.

Somewhat unexpected, we found that even participants who were unaware of the contingencies based on expectancy ratings, showed an attentional bias to the stimuli in the same direction as aware subjects. These findings suggest then that certain mechanism(s) (perhaps prediction error) may guide attentional processes implicitly and outside of conscious awareness.

In addition to expectancy ratings and observation time varying as a function of how well the cue predicted the presence or absence of reward, subsequent emotional ratings of the cues were as expected. Thus, subjects' pleasantness ratings were highest for stimulus A (which predicted reward 90% of the time), lowest for stimulus C (which predicted reward 10% of the time i.e., the absence of reward 90% of the time) with stimulus B yielding intermediate ratings. Likewise, anxiety ratings followed a similar (but mirror-imaged) pattern. Note that the "ubiquitous" stimulus X acquired similar

emotional significance as stimulus B as they both denote a 50% contingency with the outcome.

Performance transfer

More critically, the present study demonstrates that the current value of reward is important for the strength by which a referent cue can increase instrumental performance to obtain that same reward.

In the control condition (i.e., when reward value was maintained), the number of instrumental responses during each trial of the transfer test, increased linearly in accordance with the expectancies of the outcome learned during Pavlovian discrimination training, such that AX>BX>CX, both for instrumental performance (responses) and expectancy ratings. Taken together these data support our previous findings (Trick, et al., 2011) to demonstrate that the Pavlovian-instrumental transfer effect is related to explicit knowledge of the probability/uncertainty of the rewarding outcome.

Moreover, we now demonstrate that when reward was devalued by allowing participants to earn a greater (high-win) amount of money reward (relative to the task)**,** the instrumental performance was markedly reduced and the transfer effect was abolished. Additionally, when subjects were experienced with winning a much lower amount, overall instrumental performance was elevated in comparison to either control or high-win conditions and the transfer effect was also absent in this condition. Thus it seems that when the reward value becomes reduced it can also abolish the differential response to cues by leading to an overall increase in behavioural output, perhaps in an attempt to increase gains “at any cost” (i.e. even in the presence of the stimulus associated with the absence of reward – stimulus C).

However, the expectancy ratings with regard to reward outcome in each trial remained unchanged across the different revaluation conditions indicating that explicit knowledge of the outcome does not influence the behaviour under conditions when the reward value has changed. Similarly, attentional processing of the predictive cues seemed also to take place irrespective of the current reward value of the outcome. Thus it seems that the current value of the reward only modulated the instrumental performance for reward.

Taken together then the results suggest that the transfer performance effect relies on the current value of the reward outcome and that this can be dissociated from the knowledge of the outcome the referent cues predict and from the attention allocated to them (Trick, et al., 2011). These data suggest that the current reward value is a critical contributor to reward-seeking behaviour and that the predictive cues under such conditions of reward value changes lose the power to motivate behaviour, as shown here in the control condition and in previous studies where the current value of the reward was not changed (Hogarth, et al., 2009 ; Hogarth, et al., 2007).

Our data contradict those from animal studies previously showing that PIT is immune to devaluation manipulations (e.g. Holland, 2004) but are in accordance with some previous research in humans using devaluation techniques in PIT (Allman, et al., 2010). Perhaps most critically, our findings seem inconsistent with Hogarth & Chase (2011) who, like us, used abstract stimuli to train Pavlovian associations with the reinforcer outcomes in the lab. However, while we used novel stimuli, Hogarth & Chase (2011) used pictures of smoking and chocolate, which have naturally undergone extensive Pavlovian training. It is possible that such strong associations with certain outcomes in the case of smoking- and chocolate pictures are activating habit-like responses to obtain the reinforcer that are more resistant to devaluation manipulations.

It is interesting that the attentional processing (observing time) of the stimuli was unaffected by the changes in outcome value, whilst remaining sensitive to the differences in predictability of the outcomes (Mackintosh, 1975; Pearce & Hall, 1980). The dissociation between attentional processing and behavioural performance is in line with our previous study showing that blocking attention allocation to conditioned stimuli does not influence the instrumental response to receive the reward predicted by this stimulus (Hogarth, Dickinson, Janowski, et al., 2008)

These results provide convergent evidence that the mechanisms underlying attention allocation to reward-associated cues are psychologically dissociable from those involved in the motivation to seek the reward. Recent findings in rats demonstrate that they are neurobiologically separable as well. Thus, Flagel et al. (2010) using fast-scan cyclic voltammetry, studied the pattern of dopamine signalling in the nucleus accumbens of rats that developed either sign-tracking or goal-tracking responses in a lever-based Pavlovian ('autoshaping') task. Whilst dopamine was closely linked with the expression of sign-tracking performance, goal-tracking was not. Thus, much in line with the present findings, the authors were able to demonstrate a dissociation (at the neurobiological level and in the form of dopamine) between the predictive, attention-grabbing qualities of conditioned, reward-predicting cues, and their ability to incentivise performance. In light of our results showing sensitivity of transfer performance, but not attentional bias, to manipulations of value, a prediction is that cue-motivated sign-tracking, but not discriminative goal-tracking performance is dependent on and sensitive to dynamic changes in outcome value.

In conclusion, our findings provide novel insights into the psychological underpinnings of motivated performance and specifically, the role of learned Pavlovian (incentive) cues in modulating or 'spurring-on' instrumental actions in humans. Such insights are

not only relevant to understanding ‘every day’ motivation in humans, but also for understanding human conditions where, motivational processing goes awry as in the case of drug addiction. Though at odds regarding the nature of the underlying motivational or emotional states, most contemporary (neuro)psychological theories of addiction (e.g. Everitt & Robbins, 2005; Koob & Le Moal, 1997; T. E. Robinson & Berridge, 1993, etc) agree and emphasize that Pavlovian associative learning processes contribute to the maintenance of drug taking and relapse susceptibility, and therefore provide a fruitful target for intervention strategies. The emerging evidence, including the present findings, go some way towards making the point that whilst understanding the mechanisms that lead to associative memories being formed is of value, additional and better understanding of the consequences for behaviour is essential.

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Chapter 5

Paper 3: Does positive mood enhance the incentive salience of conditioned stimuli?

Abstract

Rationale The association between mood and motivation remains an important question. In particular, the effects of positive mood are less well understood and are, in this study, examined in a general Pavlovian-to-Instrumental transfer design in the presence of aversive outcomes.

Objective The aim of the present study was to investigate the effects of experimentally induced positive mood on the likelihood and vigour of an instrumental response that is trained to lead to an avoidance of a predicted aversive outcome.

Materials and methods Forty-two healthy volunteers took part in the study and split into neutral or positive mood induction conditions. A Pavlovian training schedule was used in which three compound stimuli AX, BX, CX predicted an outcome of an aversive noise on 90%, 50% and 10% of presentations respectively. This was followed by a mood induction procedure, a short phase of instrumental training and finally, transfer performed under nominal extinction.

Results Responses recorded by aware participants for both expectancy ratings and observation times for AX, BX and CX trials were matched for both experimental groups during Pavlovian training (prior to the mood induction procedure). Self-reported mood was successfully manipulated and observational patterns and expectancy ratings towards the stimuli remained unchanged by the mood condition into the transfer test. During the transfer test a significant stimuli by condition interaction was observed ($F(3, 90) = 3.96$, $\eta^2 = .117$, $p < .05$), such that participants who underwent positive mood induction produced differential responses (reduced responses in AX trials and increased

responses during CX trials) to those in the neutral mood condition, reducing the PIT effect.

Conclusions Positive mood state differentially affects observational/attentional and motivational responses to predictive Pavlovian stimuli.

Introduction

Through associative learning mechanisms, representations can be formed between stimuli in the environment and reward/aversive outcomes (Everitt, et al., 2003; Everitt & Robbins, 2005); this is often preceded by an instrumental response (Hogarth, et al., 2005; Hogarth, Dickinson, Hutton, Elbers, et al., 2006) to gain reward or avoid punishment and once a predictive associative relationship is established the stimuli become able to influence the instrumental response (Hogarth, et al., 2005). As such the stimuli in the environment acquire incentive properties (Everitt & Robbins, 2005), attract attention and drive the seeking/avoidance behaviour (Hogarth, et al., 2005). This association is often studied utilising Pavlovian-to-Instrumental transfer (PIT) (i.e. Allman, et al., 2010; Corbit, Janak, & Balleine, 2007; Hogarth, Dickinson, Hutton, Bamborough, et al., 2006; Talmi, et al., 2008; Trick, et al., 2011) as in the present study.

Both learning and motivational theories attempt to describe the underlying effect of the stimuli on seeking behaviour. Learning theories suggest that the presence of the stimuli activates mental representations of the outcome and thus drives the behaviour (e.g. Hogarth, et al., 2005; Hogarth, Dickinson, Hutton, Bamborough, et al., 2006; Hogarth, Dickinson, Hutton, Elbers, et al., 2006, etc); in contrast motivational theories suggest the presence of the stimuli increases motivation more generally and thus drives the instrumental behaviour (Everitt, et al., 2008). The interaction between mood and learning (Finger, et al., 2007), motivation (Cools, et al., 2005) and behaviour (Cools, Roberts, et al., 2008) is well established, and previous studies in our laboratory have demonstrated an effect of induced negative mood on PIT most likely through these mechanisms. The present study was interested in the effect of, the lesser-studied, induced state positive mood on motivation and attention.

Various studies in which attentional bias for both certain and uncertain stimuli has been monitored show that contingency knowledge is required for the stimuli/outcome association to be learned (Hogarth, et al., 2005). Expectancy must also be coupled with an appetitive emotional response that is indicative of the biological value of the outcome (Hogarth & Duka, 2006). However, it has also been demonstrated that a positive attentional bias for the stimulus is not essential for establishment of an instrumental response (Hogarth, Dickinson, Janowski, et al., 2008) and thus an impulsive aspect could be considered. As negative mood has long been implicated in impulsive behaviours (Clark, et al., 2005; L. Clark, et al., 2001; Dayan & Huys, 2008; O. Robinson, et al., 2009) it would be interesting to observe the effects of positive mood on impulsivity, and therefore the present study aimed to address this. The aforementioned studies demonstrate a level of uncertainty in the way in which attentional bias is affected by increasing knowledge of the outcome of the predictor (Hogarth, Dickinson, et al., 2008 ; Hogarth, Dickinson, Janowski, et al., 2008). However, the role of expectancy appears more defined and the effect of risk and ambiguity may also play a role in learning (Schultz, et al., 2008). It seems possible that expectancy and attentional bias may not be directly linked (Hogarth, Dickinson, et al., 2008) and it is possible to demonstrate this dissociation using PIT (Trick, et al., 2011). It is interesting to study the effect positive mood may have on this phenomenon, and therefore further investigate the link positive mood may have on these associations in the naturalistic environment. It does, however, seem clear that it is the expected outcome, rather than a mental representation of the outcome that drives the behaviour and thus if induced positive mood disrupts expectation of that same outcome the PIT effect may also be affected. In certain individuals, altering the value of the outcome has limited effect on instrumental behaviour and despite negative outcomes of this

behaviour compulsive seeking occurs (Economidou, et al., 2009; Everitt & Robbins, 2005), it is also possible this may be observed under condition of induced positive mood. Ultimately, as PIT is often used to study addiction (Everitt & Robbins, 2005) we aim to further understand the effect positive mood may have on such processes and investigate the link between positive mood and addiction.

A Pavlovian training schedule was used in which three compound stimuli AX, BX, CX predicted an aversive noise on 90%, 50% and 10% of presentations respectively approximating the design of Hogarth, Dickinson, et al (2008). After Pavlovian training had been completed a visual (Smith, et al., 2005) and musical mood induction procedure [adapted from Robinson, et al., (2009)] was carried out. In the transfer stage that followed, under nominal extinction, the ability of the stimuli to elicit the instrumental response was determined to study the PIT effect under different mood states. In addition, participants were able on each trial, throughout all stages, to view the stimuli for an infinite duration as determined by holding down a key; this observation time was used as a measure of attention towards each compound. It is anticipated that observation time will be proportional to the uncertainty of the stimuli, such that greatest attention will be paid to BX compared to the certain compounds AX and CX. In contrast, it is believed that the transfer effect will be AX>BX>CX and that this effect will be abolished under positive mood state. It is unclear how, or if, positive mood will affect observational patterns to stimuli associated with aversive outcomes.

Materials and Methods

Participants

Healthy subjects who were taking no medication, as determined by a medical health questionnaire were recruited. Ethical approval was obtained from the University of Sussex ethics committee and all subjects gave written informed consent prior to participation and advised they could withdraw at any time; the study was run in accordance with the Declaration of Helsinki. All participants were fully debriefed at the end of the session and compensated for their time with £15.

Forty-two subjects (18 males) took part in the study, 8 females and 2 males were excluded due to failure to successfully learn stimulus-outcome contingencies. The remaining thirty-two subjects, deemed "aware", were divided into neutral mood (n = 16; 8 females) and positive mood (n = 16; 8 females) conditions.

Experimental procedure

Subjects were asked to attend the laboratory on one occasion. They were instructed to abstain from alcohol for twelve hours prior to the testing session. In addition they were asked to avoid consuming anything high in caffeine immediately before the test session. Participants were informed they would be compensated for their time.

Materials

The task was presented on a 20" Dell P1130 monitor (Dell Inc, Berkshire, UK) and programmed using E-prime v1.1 software (Psychology Software Tools Inc.; Pittsburgh, PA). The 4 visual stimuli used (see figure 1) were black patterns displayed on a grey background, 10.2cm squared at a resolution of 1280 x 1024. Text font and size was Times New Roman 25pt. The auditory music (for the mood induction procedure and aversive noise) was played through Sennheiser PX200 headphones. Participant responses were collected via a Cherry (Pleasant Prairie, WI) mini keyboard throughout

with the top row of number keys labelled in green from 1 – 9, and the shift and space keys also labelled.

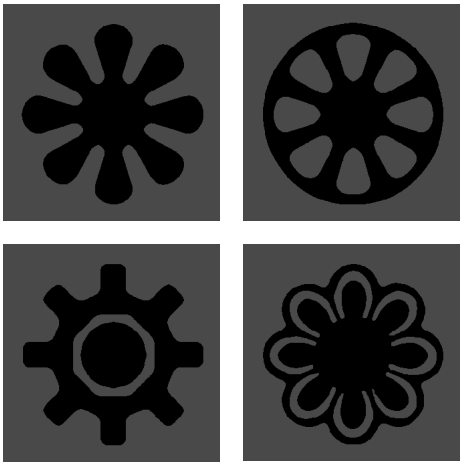


Figure 1: *Materials used during the computerised task throughout the PIT procedure; the four visual stimuli assigned to A, B, C and X in a counterbalanced fashion*

Procedure

The procedure was adapted from one previously described in Trick, et al. (2011).

Pavlovian Training: During initial Pavlovian training four visual stimuli (A, B, C and X; figure 1) were combined into three stimulus pairs, which constituted the trials (AX, BX, CX) and which predicted the occurrence of an aversive noise with a 90%, 50% or 10% probability respectively, approximating the design of Hogarth, Dickinson, et al. (2008). The stimuli were presented in compound with the common stimulus X (Wagner, 1969) in order to assess selective attention for the concurrently presented stimuli A, B and C,

which were informative of the trial outcome. The four visual stimuli shown in figure 1 were assigned to the role of A, B, C and X in counterbalanced order across participants.

Each trial started with a fixation cross in the centre of the screen. Once the participant pressed the space bar the fixation cross turned yellow. At this point participants pressed and held the shift key, which terminated the cross and presented a stimulus pair, with the cues 10.5cm either side of the location previously occupied by the fixation cross. The stimulus-pair remained on screen as long as the shift key was held and this represented the **observing time**. Once the shift key was released the stimulus-pair vanished, and the expectancy question "How likely is the loud noise 1 = unlikely 5 = don't know 9 = likely", was shown in the centre of the screen. Participants answered this question by pressing a green number key between 1 and 9 providing outcome **expectancy ratings** and the question disappeared. Following this, the screen displayed only the grey background for 5 sec and during the last 4 sec of this time aversive noise (40msec 97dB) could occur at any randomly selected millisecond. The training phase consisted of 120 trials, arranged in 2 blocks of 60 trials[1].

Mood Induction: The procedure was adapted from one previously described (O. Robinson, et al., 2009). Participants were presented with 44 (positive or neutral) pictures (Smith, et al., 2005), whilst music was played through Sennheiser PX200 headphones. Participants were instructed to get as deeply as possible into any mood

[1] Trials within the block were randomised for type (AX, BX, CX) and stimulus location (left, right) Critically, the outcome occurred in 90% of AX trials (18/20), in 50% of BX trials (10/20) and 10% of CX trials (2/20). Stimulus location was balanced within trials with and without the outcome.

evoked. Firstly, a blank screen was presented and participants were instructed to press the space bar to view the first picture. The picture was displayed in the centre of the screen for 12 seconds, immediately followed by a blank screen, and participants were asked to look at the picture for as long as it was displayed. When the blank screen was displayed again participants pressed the space bar to view the next picture. The music played was *Serenade No.13 KV 525 G Major: I. Serenade. Allegro* by Wolfgang Amadeus Mozart for the Positive version of the MIP and for the neutral MIP: *The Planets, Po. 32: VII. Neptune, the Mystic* by Gustav Holst. Prior to and after the MIP, a set of Visual Analogue Scales (VAS) were administered, to determine self reported mood. Comparison between initial- and post- MIP self reported mood, using VAS, was used to determine the **mood effects of the MIP** and therefore assess the effectiveness of the procedure.

Instrumental training: Participants were then trained to acquire an instrumental response (spacebar pressing) in a procedure identical to Pavlovian training apart from the following modifications. First, holding down the shift key presented two blank grey squares in place of the compounds used in Pavlovian training. Participants were instructed that repeatedly pressing the spacebar during the interval following the expectancy question would sometimes lead to prevention of the noise. The loud noise was scheduled to occur automatically on 25% of trials, and on a further 25%, which were avoidable by performing a key press within the 1-sec window leading up to the scheduled time of the outcome (thus, participants best strategy was to respond at least once per second across the period following expectancy question). Consequently, 25% of trials were accompanied by the outcome automatically. There were 8 trials of this simple instrumental training.

Pavlovian to Instrumental Transfer: The transfer phase followed the design of the instrumental phase except that compounds established in Pavlovian training (AX, BX, CX) were presented randomly intermixed with blank trials of instrumental training, with equal proportions (16 trials each), over 64 trials. The noise outcome was scheduled for 25% of all trials, with a further 25% possible if instrumental responding was not performed effectively (as for instrumental training). Thus the Pavlovian contingencies established in training were not in force in the transfer phase. The number of instrumental responses (space bar presses), made during the variable time window prior to the scheduled time of the noise, were recorded to determine the **transfer effect**. This variable time window was matched for trials in which the outcome (noise) was and was not scheduled.

Evaluative conditioning

At the end of the task, the affective evaluation of stimuli was recorded to provide an alternative assay of conditioning. Participants were presented with the individual stimuli A, B, C, and X, in random order, and answered the questions "How anxious does this picture make you?" and "How pleasant do you find this picture?" in random order, on a scale from 1-9 where 1 = not at all, and 9 = extremely. The affective responses were examined in relation to the impact of cues on attention and instrumental performance.

Statistical analysis

Data were analysed independently for each study and separately for Pavlovian training and transfer sections within each study, and also for aware and unaware participants.

Initial analysis was performed using a 2x3x2 mixed ANOVA with the between factor Condition (2 levels – Positive Mood, Neutral Mood), and within factors Trial (3 levels - AX, BX, CX) and Block (2 levels – block 1, block 2) for the variables Expectancy

ratings and Observing times. The Block and Condition variables were eventually collapsed for Expectancy ratings and Observing times because they showed no interesting effects or interactions, to simplify reporting of the key findings. This was followed by post-hoc Bonferroni where appropriate unless otherwise stated.

Analysis of the PIT effect, assessed using number of instrumental responses, was analysed as above with the exception of the factor Trial, which now had four levels (4 levels - AX, BX, CX, Blank) by inclusion of the blank. Other analysis was performed using mixed ANOVA followed by Bonferroni post-hoc where appropriate unless otherwise stated. A Greenhouse-Geisser correction was used where required throughout.

Results

Biographical data were collected from both aware and unaware participants and demonstrated no significant differences between conditions.

Awareness of training contingencies

In the final half of Pavlovian training (60 trials) each participant produced 20 expectancy ratings for each of the three trial types; AX, BX and CX. For each participant the three trial types were compared in the second block of trials using a one-way, within-subjects ANOVA. If there was a significant main effect of trial type, and the direction of effect was veridical with the scheduled Pavlovian contingencies (i.e., AX>BX>CX) the participant was labelled ‘aware’, otherwise the participant was labelled ‘unaware’. The awareness criteria bisected participants who ranged on a continuum of predictive knowledge, rather than participants falling on a step function of predictive knowledge (Lieberman, et al., 1998). The awareness criteria therefore isolate participants who achieved the greatest predictive knowledge in the training

provided. The aware group was analysed independently to confirm the co-occurrence of predictive knowledge and conditioned responding (Hogarth & Duka, 2006 ; Lovibond & Shanks, 2002), data for unaware participants is shown tabulated (table 1).

Table 1

Data from unaware participants –

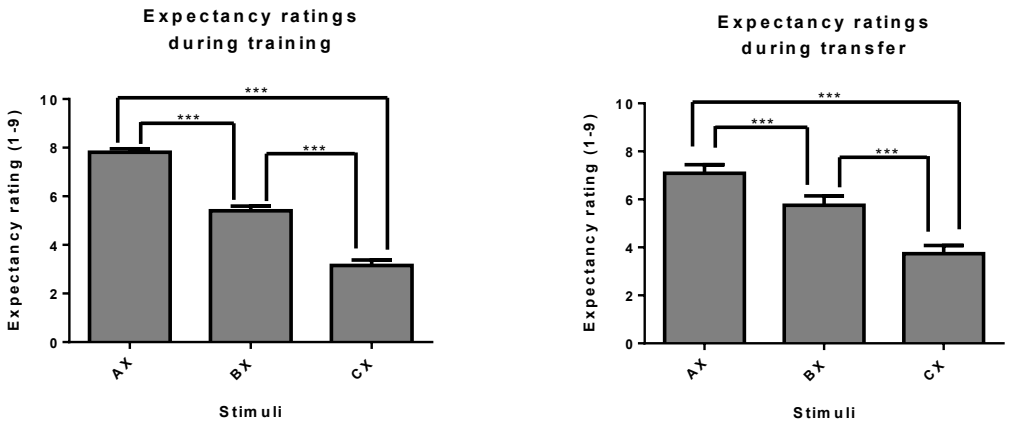
Mean and standard error (SEM)

(split by induced mood condition)	**Mean**		**SEM**	
	Positive (n=1)	**Neutral (n=9)**	**Positive**	**Neutral**
Pavlovian Training Phase				
Training Expectancy (1-9) – AX	7.33	6.43	-	0.31
Training Expectancy (1-9) – BX	3.13	6.15	-	0.24
Training Expectancy (1-9) – CX	5.03	4.40	-	0.46
Training Observing times (ms) - AX	1139.40	3015.24	-	510.73
Training Observing times (ms) - BX	1359.28	2914.99	-	430.39
Training Observing times (ms) - CX	1297.57	2801.41	-	418.66
Transfer Phase				
Transfer Expectancy (1-9) – AX	6.81	5.92	-	0.47
Transfer Expectancy (1-9) – BX	4.75	6.04	-	0.83
Transfer Expectancy (1-9) – CX	5.19	4.93	-	0.40
Transfer Observing times (ms) - AX	586.00	2027.47	-	454.35
Transfer Observing times (ms) - BX	663.94	1477.47	-	333.62

Transfer Observing times (ms) - CX	1023.00	1949.63	-	356.29
Transfer number of responses – AX	16.19	10.53	-	1.98
Transfer number of responses – BX	11.69	12.28	-	2.18
Transfer number of responses – CX	14.06	9.78	-	2.07
Transfer number of responses - Blank	16.56	14.85	-	1.11
Evaluative conditioning				
Anxiety rating (1-9) – A	8.00	4.33	-	0.91
Anxiety rating (1-9) – B	1.00	3.33	-	0.96
Anxiety rating (1-9) – C	3.00	4.00	-	0.85
Anxiety rating (1-9) – Blank	1.00	4.56	-	0.78
Pleasantness rating (1-9) – A	1.00	5.11	-	0.86
Pleasantness rating (1-9) – B	8.00	5.89	-	1.10
Pleasantness rating (1-9) – C	4.00	5.56	-	1.00
Pleasantness rating (1-9) – Blank	9.00	3.11	-	0.48

Expectancy ratings

Figure 2 shows the expectancy ratings for AX, BX and CX trials during training for aware participants. Aware participants recorded significantly different ratings for each stimulus, $F(1.63, 50.63) = 129.40$, $\eta^2 = .807$, $p < .001$, but the unaware group did not ($F(1.21, 9.64) = 4.30$, $\eta^2 = .350$, $p = .061$). These effects were determined by the pre-selection of aware and unaware groups. These differential expectancies were maintained into transfer (shown in figure 2), such that in the aware group, there was a significant main effect of trial type, $F(2, 62) = 33.66$, $\eta^2 = .521$, $p < .001$. The unaware group showed no main effect of trial type ($F(2, 18) = 1.91$, $\eta^2 = .175$, $p = .177$). These results were consistent for all experimental groups and the data for aware participants were therefore collapsed.

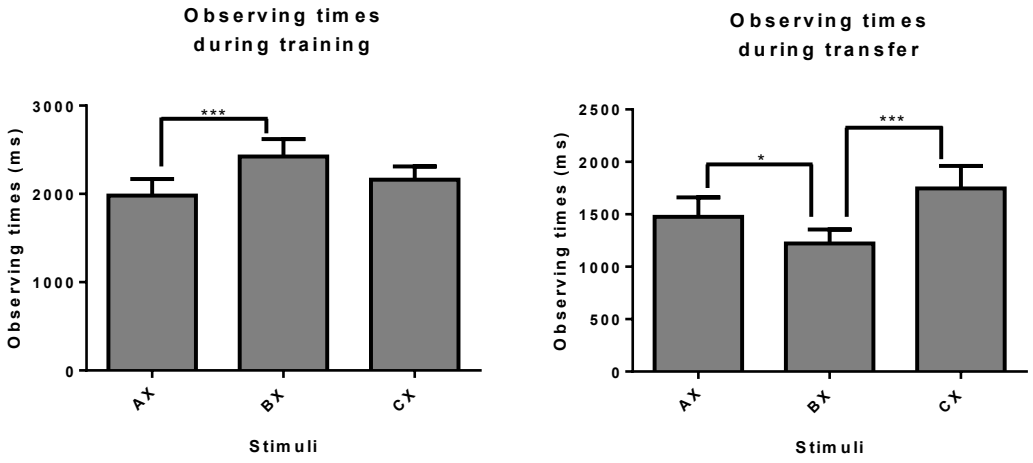


(a) **(b)**

*Figure 2: Mean expectancy ratings following the presentation of trials AX, BX, CX during (a) Pavlovian training and (b) Transfer phase for aware participants. No differences were observed between the conditions and the data were therefore collapsed generating n=32 per bar graphically represented. Error bars represent standard error (SE) of mean. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

Observing time

Figures 3a and 3b show observing times (ms) during Pavlovian training and transfer. In aware participants, there was a significant main effect of trial type, $F(1.62, 50.42) = 8.51$, $\eta^2 = .215$, $p < .005$, with observing times being overall longest in BX trials compared to AX and CX trials. There was no significant trial effect for unaware participants during training ($F(2, 18) = .441$, $\eta^2 = .047$, $p = .650$), or transfer ($F(2, 18) = 1.97$, $\eta^2 = .180$, $p = .168$). By contrast (for aware participants), during transfer, BX observing times were shorter compared to AX and CX and there was a significant main effect of trial type for aware participants, $F(1.62, 50.10) = 12.42$, $\eta^2 = .286$, $p < .001$, this result is consistent with the results of Hogarth, Dickinson, et al. (2008) and further demonstrates that observing time can be used to index the predictive uncertainty of stimuli; this pattern was consistent for all experimental groups and the data for aware participants were therefore collapsed.



(a) **(b)**

*Figure 3: Mean observing times (ms) for trials AX, BX, CX during the Pavlovian training (a) and the transfer phase (b) for aware participants. No differences were observed between the conditions and the data were therefore collapsed generating n=32 per bar graphically represented. Error bars represent SE of mean. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.*

Mood effects of the MIP

The effects of mood were calculated by determining the difference in response to VAS before and after the MIP, for aware participants. There was a significant overall change in mood by condition interaction ($F(1, 30) = .926$, $\eta^2 = .030$, $p < .005$). This effect is explained by those in the positive condition reporting a greater decrease in sadness (mean: -0.50; SEM: 0.27) after the MIP than those in the neutral condition (who actually showed a increase – mean: 1.63; SEM: 0.70) (p=0.011) and also generating a

greater increase in happiness (mean: 1.00; SEM: 0.52) after the MIP than those in the neutral condition (who showed a decrease – mean: -1.06; SEM: 0.52) (p=0.009).

Transfer effect

Figure 4 shows the number of key press responses produced in AX, BX, CX and blank trials for aware participants respectively for each condition. For aware participants there was a significant stimulus effect $F(3, 90) = 13.76$, $\eta^2 = .314$, $p < .001$, indicating a PIT effect. There was also a significant stimulus by condition interaction: $F(3, 90) = 3.96$, $\eta^2 = .117$, $p < .05$, explained by those in the positive mood condition reducing responses in AX trials, but increasing responses in CX trials compared to the neutral condition. These effects were not observed for the unaware participants, $p > .511$.

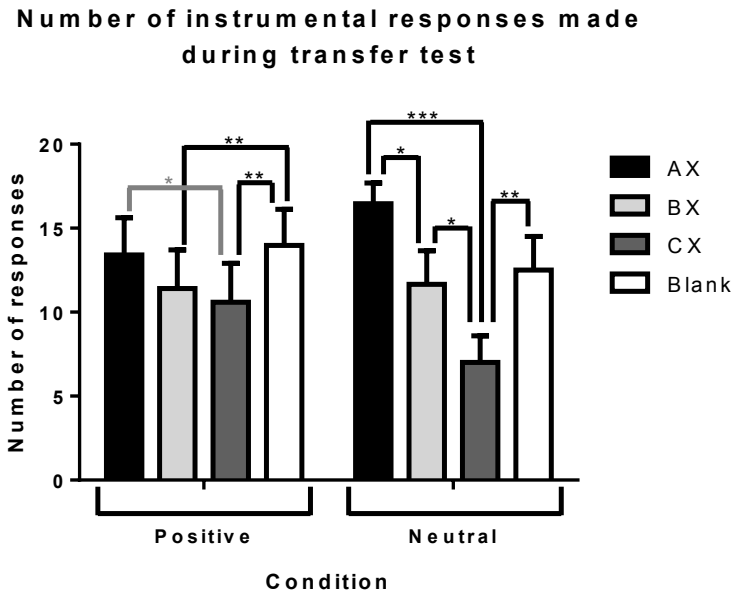


*Figure 4: Mean instrumental responses following presentation of trials AX, BX, CX and blank trials during the Transfer phase for aware participants for each condition (n=16 per condition). Error bars represent SE of mean. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

Evaluative conditioning

Tables 1 and 2 show the pleasantness and anxiety ratings to stimuli reported at the end of the task for unaware and aware participants respectively. For aware participants, there was a stimuli by condition interaction for anxiety ratings, $F(2, 60) = 3.97$, $\eta^2 = .117$, $p < .05$, on exclusion of the "X". No significant effects with regard to anxiety ratings were recorded for the unaware group ($p > .164$). No significant effects or interactions were observed for pleasantness ratings for either aware ($F(3, 93) = 1.42$, $\eta^2 = .044$, $p = .242$), or unaware participants ($F(3, 27) = 1.15$, $\eta^2 = .113$, $p = .349$).

Table 2

Mean emotional ratings (SEM)

made by aware participants

Measurement	**Stimuli**			
Study/Condition	**A**	**B**	**C**	**X**
Anxiety ratings				
Positive mood condition mean	3.69	4.38	3.94	3.38
	(0.72)	(0.69)	(0.73)	(0.66)
Neutral mood condition mean	6.31	4.93	3.56	4.43
	(0.70)	(0.61)	(0.59)	(0.59)

Pleasantness ratings				
Positive mood condition mean	5.31	4.56	3.69	5.19
	(0.64)	(0.54)	(0.60)	(0.65)
Neutral mood condition mean	5.25	5.06	4.69	5.44
	(0.71)	(0.59)	(0.76)	(0.35)

Discussion

The present study explored whether induced positive mood would disrupt previously learnt Pavlovian (S-O) conditioned associations and/or transfer of a Pavlovian cue's motivational effects to instrumental (A-O-mediated) performance. Importantly, a positive mood state was successfully induced in participants in the positive mood condition as assessed using self-reported VAS.

Evaluative Conditioning

As reported previously (Austin & Duka, 2010, 2012), the present results show that successful Pavlovian training generated differential expectations about the probability of the aversive outcome in AX, BX and CX trials. Specifically, both during the Pavlovian training phase and the transfer test (when these contingencies were no longer reinforced), decreases in the predictability of the aversive outcome from 90%, to 50% and 10% produced a greater decline in expectancy rating in subjects deemed 'aware' of the contingencies. Additionally, attention to the cues (observing times) was linked with the level of uncertainty (Pearce & Hall, 1980), such that during Pavlovian training, observing times were greatest during BX stimulus trials (the 50% uncertain predictor)

compared to stimulus trials that were highly predictive of the outcome (90%) or not (10%). In contrast, during transfer observing times were reversed (relative to training) such that attention to BX was reduced below the levels during AX and CX trials. These results in fact agree with our previous data (Trick, et al., 2011), suggesting that the decrease in observing time during BX trials might be related to prediction error.

Transfer Performance

More critically, data from the present study support our previous findings (Trick, et al., 2011) that the explicit knowledge of the probability/uncertainty of the outcome is important for the strength by which a referent cue can increase instrumental performance to avoid that same outcome. In the control condition (neutral mood), the number of instrumental responses during each trial of the transfer test, increased linearly in accordance with the expectancies of the outcome learned during Pavlovian discrimination training, such that AX>BX>CX, both for instrumental performance (responses) and expectancy ratings.

Moreover, we now demonstrate that when under state positive mood (induced by a musical and visual positive mood induction procedure) the instrumental performance was affected and a suppression in the transfer effect observed i.e. when experienced with induced positive mood participants did not show the same good discriminative response produced by those in the neutral condition. Thus it seems that when the participants current mood is manipulated to become positive it can also reduce the differential response to cues by leading to an overall increase in behavioural output for stimulus C, perhaps in an attempt to avoid the aversive outcome under any circumstance (i.e. even in the presence of the stimulus associated with the absence of the aversive noise – stimulus C). However, the expectancy ratings with regard to aversive outcome

in each trial remained unchanged in all mood conditions indicating that explicit knowledge of the outcome does not influence the instrumental behaviour. Similarly, attentional processing of the predictive cues seemed also to take place irrespective of the current state mood of the participant. Thus it seems that induced mood only modulated the instrumental performance.

Taken together then, the results suggest that the transfer performance effect can be modulated by the current mood state of the participant and that this can be dissociated from the knowledge of the outcome the referent cues predict and from the attention allocated to them, supporting previous findings (Trick, et al., 2011). There are two possible explanations for this observation. Firstly, these data could suggest that state mood can manipulate the perceived current value of the outcome, as we have previously demonstrated that this is a critical contributor to instrumental/seeking behaviour and that the predictive cues under such conditions of value changes lose the power to motivate behaviour when the value is actually altered (Mathers, Crombag, Steckler, & Duka, 2014); additionally this was also the case in previous studies where the current value of the reward was not changed (Hogarth, et al., 2009 ; Hogarth, et al., 2007). The alternative possibility would be that the attenuated PIT effect observed in the positive mood condition is driven by an general alteration in motivation, such that the number of instrumental responses in CX trials is increased to be more in line with BX and AX trials as supported by previous data in negative mood studies whereby induced state mood modulated motivation (Mathers, Steckler, & Duka, 2014). Interestingly, the aforementioned study on negative mood demonstrated a dissociation between punishment and reward prediction, such that the PIT effect was not abolished using a punishment outcome as was the case when the outcome was reward. The current data provide further support for the latter findings, in that the PIT was not completely

abolished albeit reduced. Support for these data comes further from studies, which have shown punishment prediction to be resistant to mood changes, whereas reward prediction is more susceptible to mood manipulations (Cools, Robinson, et al., 2008; Daw, et al., 2002).

The current study did not aim at separating between a general motivational state that the mood manipulation had generated, and the value of the reinforcer. Since responses did not increase across all stimuli but specifically for the CX in the positive mood state, we could postulate that in the current study the negative value of the CX was changed to appear more positive. This may be specific to an aversive procedure as data from an appetitive procedure are lacking. However, in a study in which we increased the value of the reinforcer in an appetitive paradigm responses were increased across all stimuli indicating more a general increase in motivation (Mathers, Crombag, et al., 2014). Measurements of attention in the present study remained unaffected by the mood manipulation. This was also the case when changes to outcome value were made in a previous devaluation study (Mathers, Crombag, et al., 2014) where attention to stimuli remained unaffected by the new value given to the outcome. Attention to the stimuli remained sensitive to the differences in predictability of the outcomes (Mackintosh, 1975; Pearce & Hall, 1980) as also observed in the present data.

The results of the current study therefore provide further evidence that the mechanisms underlying attention allocation to outcome-associated cues are psychologically dissociable from those involved in the motivation to seek the outcome (reward/aversive outcome avoidance), as well as being neurobiologically separable as well (Flagel, et al., 2010). This is further supported by previous studies (Clark, et al., 2001) which apportion the role of mood to be at the neurobiological level.

Our data are also interesting with regard to the concept of "depressive realism" which describes that perception of causal control is more accurate in depressive states (Chase, Michael, Bullmore, Sahakian, & Robbins, 2009; Chase, et al., 2010). According to this theory participants under an induced negative mood state during PIT will acquire the new contingencies more quickly (i.e. will extinguish faster) and therefore will not show a PIT effect, as the reduced discrimination is actually a more accurate response as the outcome contingencies were no longer in force in the transfer test and all stimuli were equally predictive of the outcome. From our data we could suggest that positive mood may also facilitate extinction as PIT was reduced in this condition compared to the neutral mood.

In conclusion, our findings provide further insights into the effects positive mood has on motivated performance and the underpinnings of instrumental actions in humans. This is important for our understanding of abnormal behaviours involving motivation, such as drug addiction, and may therefore provide a target for intervention strategies.

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Chapter 6

Paper 4: The impact of positive and negative mood on the speed and accuracy of acquiring Pavlovian contingencies of stimuli predictive of reward

Abstract

Rationale Negative mood has long been implicated in learning but there is still some debate over the exact mechanisms of this interaction.

Objective The aim of the present study was to investigate the effects of experimentally induced negative and positive mood on a particular example of learning: acquisition of Pavlovian contingencies, predictive of a reward, during Pavlovian training, and to assess the extent to which state mood may alter speed of learning, emotional responses to stimuli predictive of reward and attention to the stimuli.

Materials and methods Forty nine healthy volunteers underwent negative (n=16), neutral (n=16) or positive (n=17) mood induction prior to participating in a Pavlovian training paradigm designed to measure learning, attention and emotional responses to stimuli. During Pavlovian training participants were trained on a Pavlovian schedule in which three visual stimuli, A, B and C, predicted the occurrence of a monetary reward (gain of 10p) with 90%, 50% or 10% probability, respectively.

Results Participants who underwent negative mood induction took longer to acquire Pavlovian contingency knowledge than those in either neutral or positive mood condition. Mood state also disrupted observation patterns to the stimuli such that induced mood reduced overall observation time in BX and CX trials, as well those in a negative mood state only showing a increased bias for stimulus C over X. Average pupil size in all trial types was increased by positive mood.

Conclusions Positive and negative mood state differentially affect emotional and observational responses to Pavlovian stimuli predictive of reward, and also impact on the rate of acquiring contingency knowledge of the same predictive stimuli.

Introduction

The effects of negative, and positive (Clark, et al., 2001), mood have long been studied, as has the effectiveness of mood induction procedures to experimentally induce a certain mood and thus manipulate state mood allowing its effects to be explored (e.g. Chase, et al., 2010; Gilet, 2008; Robinson, et al., 2009; Robinson & Sahakian, 2009b, etc). Previous studies have implicated negative mood in impulsivity (Clark, et al., 2005), instrumental learning and information processing (Finger, et al., 2007; Merens, et al., 2007), motivation (Cools, et al., 2005) as well as emotional and behavioural processing (Cools, Roberts, et al., 2008). Mood may, therefore, also have a link with addictive processes (Cools, et al., 2005). One theory proposes that mood affects motivation (Cools, et al., 2005; Cools, Roberts, et al., 2008; Cools, Robinson, et al., 2008), which in turn would impact upon addictive processes. Additionally, previous studies utilizing Pavlovian to instrumental transfer (PIT) in humans, conducted in our hands, demonstrated a reduced motivation to gain reward, and avoid punishment, when under induced negative mood (Mathers, Steckler, et al., 2014). PIT is a model that has often been used to study addiction (Everitt & Robbins, 2005). It has been widely accepted that humans are able to transfer the predictive Pavlovian stimulus-outcome relationships to independently learned instrumental responding for that same outcome (Balleine & O'Doherty, 2009; Hogarth, Dickinson, Hutton, Bamborough, et al., 2006) and it is believed that such a transfer effect is modulated by the predictive strength of the stimulus, which, in turn, exerts a motivational influence driving the instrumental responding (Everitt & Robbins, 2005). Thus our previous data provide further evidence linking negative mood and reduced motivation. Motivation is important for reward seeking, and is also required for many normal processes (Robinson & Berridge, 2003) but it is also implicated in abnormal processing and driving of undesirable behaviours.

During our previous studies we explored the effect of negative, and positive, mood on the PIT effect and carried out mood induction after Pavlovian learning had taken place. It was interesting to note that Pavlovian contingency knowledge was maintained into the transfer stage despite negative mood induction (Mathers, Steckler, et al., 2014), which did not agree with other data (Rogers, et al., 2003) which demonstrated a link between serotonin and reduced discrimination between expected gains. In order to fully understand the effect of mood on motivation, and more broadly Pavlovian learning, it is important to reconcile these discrepancies. In light of the differential effects of negative mood on reward discrimination we proposed that if mood induction was carried out prior to Pavlovian learning, the contingencies would be acquired by those under state negative mood but at a different rate than those under positive or neutral induced mood.

To test this hypothesis this study adopted the use of a musical and visual mood induction procedure (MIP) adapted from one previously described (Robinson, et al., 2009) to induce positive, negative and neutral (control condition) mood states to allow the effect of the current state of these moods on learning to be examined. This was followed by an extended session of Pavlovian learning in which participants were trained on a Pavlovian schedule in which three compound stimuli, AX, BX and CX, predicted a reward (gain of 10p) outcome with a probability of 90%, 50% and 10%, respectively approximating the design of Hogarth, Dickinson et al. (2008). Throughout the Pavlovian learning stage of the study, attention to compounds was assessed by the duration for which participants held down an "observing response" key to present these compounds (Premack & Collier, 1966), and attention to individual stimuli within the compounds was assessed by gaze dwell time measured with an eye tracker. Pupil size was also recorded.

It is anticipated that attention to the stimuli will accord with the predictive uncertainty of the stimuli. Specifically, observing times should be maximal for the partially predictive compound BX compared to the reliable predictive and non-predictive compounds AX and CX. On top of this, dwell time for the single stimuli in the compound as measured with the eye tracker should be greater for the stimulus B compared to the concurrent presented common stimulus X, whereas the reliable predictive and non-predictive stimuli A and C should show smaller dwell time biases relative to the concurrently presented X stimulus. The use of the eye tracker during Pavlovian training aims to address theories concerning the role played by attention in learning (Dayan, Kakade, & Montague, 2000), decision making (Schultz et al., 2008), and therefore link to drug dependence (Field, Munafo, & Franken, 2009), and the effect mood may have on these.

Procedures were approved by the University of Sussex Ethics Committee. Participants were informed they were allowed to withdraw at any time.

Materials and Methods

Participants

49 healthy participants (25 male, 24 female) aged between 18 and 32 years (mean 21.1 years) were recruited from staff and students at the University of Sussex. All participants had 20:20 or 20:30 vision (assessed with the Snellen three-metre visual acuity test). All participants were in good health and taking no medication as determined by a medical health questionnaire. Subjects were divided into Negative Mood Group (N=16; 8 male & 8 female; mean age 20.88 years, S.D. 2.68), Neutral

Mood Group (N=16; 8 male & 8 female; mean age 20.69 years, S.D. 2.50) and Positive Mood Group (N=17 (9 male & 8 female; mean age 21.6 years, S.D. 3.94). Ethical approval was obtained from the University of Sussex ethics committee, and all participants gave written informed consent prior to participation. At the end of the experimental session participants were debriefed and paid £15.

Experimental procedure

Subjects were asked to attend the laboratory on one occasion. Participants were instructed to abstain from alcohol for twelve hours prior to the testing session. In addition they were asked to avoid consuming anything high in caffeine immediately before the test session. Participants were informed they would be compensated for their time as well as receiving any money they won on the task.

Materials

All procedures were programmed using E-Prime v1.1 software (Psychology Software Tools Inc.; Pittsburgh, PA) and presented on a 20" Dell P1130 screen. Each of four visual stimuli (see figure 1) were black patterns displayed on a grey background, 10.2cm squared at a resolution of 1280 x 1024. Text font and size was Times New Roman 25pt. The auditory music (for the mood induction procedure) was played through Sennheiser PX200 headphones. Participant responses were collected via a Cherry mini keyboard throughout, with the top row of number keys labelled in green from 1 – 9, and the shift and space keys also labelled.

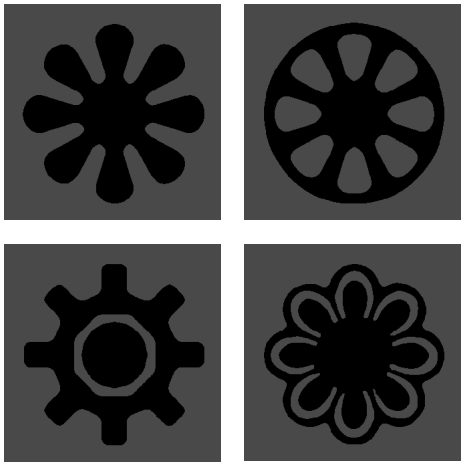


Figure 1: *Materials used during the computerised task throughout the PIT procedure; the four visual stimuli assigned to A, B, C and X in a counterbalanced fashion*

Participants wore a head mounted Eyelink II eye-tracker (SR-Research Ltd.; Ontario, Canada) for the duration of the task. The position of the eye was sampled at a rate of 500Hz. A parallel port connection linked the eye-tracker with the E-Prime program.

Procedure

Mood Induction Procedure (MIP)

The procedure was adapted from one previously described in Robinson, et al. (2009). Participants were presented with 44 (positive, negative or neutral) pictures (Smith, et al., 2005), whilst music was played through Sennheiser PX200 headphones. Participants were instructed to get as deeply as possible into any mood evoked. Firstly, a blank screen was presented and participants were instructed to press the space bar to view the first picture. The picture was displayed in the center of the screen for 12 seconds, immediately followed by a blank screen, and participants were asked to look at the

picture for as long as it was displayed. When the blank screen was displayed again participants pressed the space bar to view the next picture. The music played was *Adagio in G Minor* by Thomas Albinoni for the negative version of the MIP, *Serenade No.13 KV 525 G Major: I. Serenade. Allegro* by Wolfgang Amadeus Mozart for the Positive version and for the neutral MIP *The Planets, Po. 32: VII. Neptune, the Mystic* by Gustav Holst. Prior to and after the MIP, a set of Visual Analogue Scales (VAS) were administered, to determine self reported mood. Comparison between initial- and post- MIP self reported mood, using VAS, was used to determine the mood effects of the MIP and therefore assess the effectiveness of the procedure. It is important to note that the Beck depression inventory (BDI) was pre-administered and BDI scores (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) were recorded and the conditions were matched for trait depressiveness using the BDI, as vast variances on BDI scores have been observed in normal populations (Chase, et al., 2010).

Pavlovian training

During initial Pavlovian training four visual stimuli (A, B, C and X; see figure 1) were combined into three stimulus pairs, which constituted the trials (AX, BX, CX) and which predicted the occurrence of a reward (gain of 10 pence) with a 90%, 50% or 10% probability respectively. Throughout the paper the stimuli A, B, and C are named the informative stimuli in the pair, whereas stimulus X paired with A, B or C is named the uninformative stimulus X(A), X(B) and X(C) in the pair. The four visual stimuli shown in figure 1 were assigned to the role of A, B, C and X in counterbalanced order across participants. At the start of the task, the participant was instructed that each trial would begin with a fixation cross (+) in the centre of the screen and that when they looked at the cross directly it would turn yellow. At this point they were able to view two pictures by holding down the shift key. Then they were asked how likely they were to receive

the reward and to rate the likelihood on a scale of 1 - 9, where 1= unlikely and 9 = likely. Participants were instructed to “Press the space bar to begin”.

During each trial, participants were presented with a black fixation cross in the centre of the screen. This allowed the experimenter to calibrate the eye-tracker while the participant focused on the cross, and the fixation cross turned yellow once calibration was complete. At this point participants pressed and held the shift key, which terminated the cross and presented a stimulus pair, with the cues 10.5cm either side of the location previously occupied by the fixation cross. The stimulus-pair remained on screen as long as the shift key was held and this represented the observing time. Dwell time was measured during the time window of the observing time and was calculated separately for the informative and uninformative stimulus as a percentage of the observing time in each particular trial that gaze was fixated on the stimulus, to generate dwell time (%) scores for each stimulus. These scores quantify the percentage in fixating the informative stimulus, and to the uninformative stimulus, as a percentage of the total observing time for that trial. Once the shift key was released the stimulus-pair vanished, and an expectancy question, “How likely is the reward 1 = unlikely 5 = don't know 9 = likely”, was shown in the centre of the screen. Participants answered this question by pressing a green number key between 1 and 9 providing outcome expectancy ratings and the question immediately disappeared. Following this, the screen displayed only the grey background for 5 sec and during the last 4 sec of this time “You gain 10p” could be displayed on the screen at any randomly selected millisecond; on rewarded trials participants transferred 10p to their “My Money” tin. The reward occurred in 90% of stimulus pair AX trials, in 50% of BX trials and in 10% of CX trials. The stimulus pair displayed in each trial was randomly selected. The training phase consisted of 180 trials, arranged in 6 blocks of 30. Pupil size was measured

during the time window of the observing time and was calculated separately for the informative and uninformative stimulus, as a mean of the pupil size across all fixations to that stimulus during that trial. The measurements for the informative stimuli were averaged across all trials for that stimuli, by block, to give pupil size scores for each informative stimulus.

Evaluative conditioning

At the end of the task participants were presented with the individual stimuli A, B, C, and X, in random order, and asked "*How anxious does this picture make you feel? Press a green number key between 1 and 9 to indicate the strength of your feeling 1 = not at all anxious 9 = extremely anxious*", and "*How pleasant do you find this picture? Press a green number key between 1 and 9 to indicate the strength of your feeling 1 = not at all pleasant 9 = extremely pleasant*". Anxiety and pleasantness ratings represented the emotional response measurements.

Statistical analysis

Unless stated otherwise, all analyses were performed using repeated measures ANOVA and significant main effects were interpreted using pairwise comparisons, where appropriate, with Bonferroni-corrected post-hoc tests unless noted. An error rate of $p<0.05$ was used to define significance. The block variable was collapsed to simplify reporting of the key findings where stated. A Greenhouse-Geisser correction was used where required.

Results

Contingency awareness and learning in training

During each block of Pavlovian training each participant produced 10 expectancy ratings for each of the three trial types; AX, BX and CX. For each participant the three trial types were compared using a one-way within-subjects ANOVA. If there was a significant main effect of trial type, and the direction of effect was veridical with the scheduled Pavlovian contingencies (i.e. AX>BX>CX) the participant was labelled 'aware', otherwise the participant was labelled 'unaware'. Once 30 consecutive aware trials were achieved the participant was labelled aware and the middle trial number of these 30 was labelled as the trial number in which they had successfully reached the learning criterion (see below). Of the 17 participants assigned to the positive condition 10 ultimately become aware (5 male, 5 female), of the 16 in the negative condition 10 participants achieved awareness (5 male, 5 female) and of the 16 in the neutral condition 10 participants (4 male, 6 female) were found to eventually become aware. Given that human conditioned behaviour is strongly associated with contingency knowledge (Hogarth & Duka, 2006 ; Lovibond & Shanks, 2002), we excluded the unaware group from the main analyses unless otherwise stated.

Mood effects of the MIP

The change in self-reported mood was calculated by determining the difference in response to VAS before and after the MIP, reported by participants. There was a significant overall change by condition interaction ($F(2, 46) = 8.39$, $p=0.001$), explained by those in the negative condition reporting a greater increase in sadness after the MIP (positive mean: -.076; SEM: 0.45, negative mean: 2.00; SEM: 0.47, neutral mean: -0.44; SEM: 0.76) than those in either the positive ($p<0.01$) or neutral condition ($p<0.01$);

those in the positive condition also reported a greater increase in happiness after the MIP (positive mean: 0.35; SEM: 0.40, negative mean: -1.63; SEM: 0.46, neutral mean: -0.56; SEM: 0.47) than those in the negative condition ($p<0.05$).

Learning criterion

Figure 2 shows the mean trial number in which participants achieved the learning criterion in each condition. There was a significant effect of condition on the trial number in which participants became aware ($F(2, 27) = 3.60$, $p = .041$), such that those in the negative condition took longer to reach the learning criterion than those in the positive condition ($p<.05$).

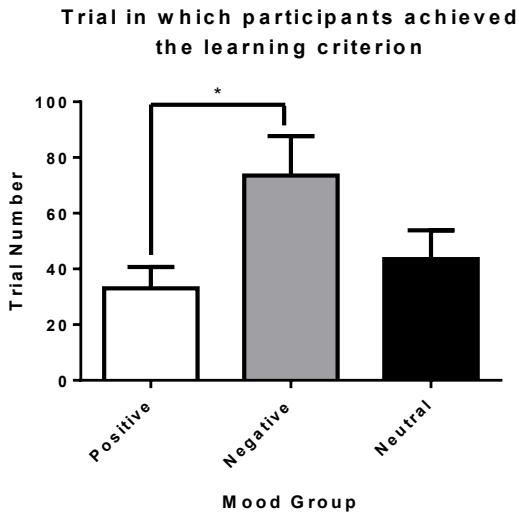
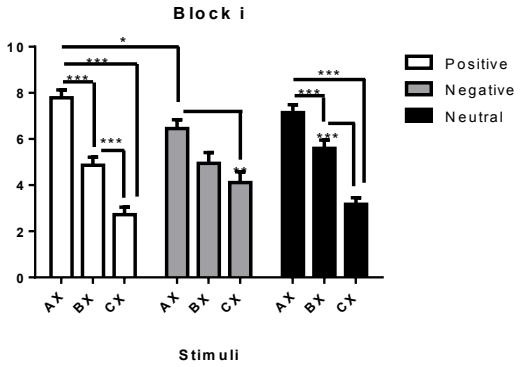


Figure 2: *Mean trial number in which participants in each condition achieved the learning criterion; * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

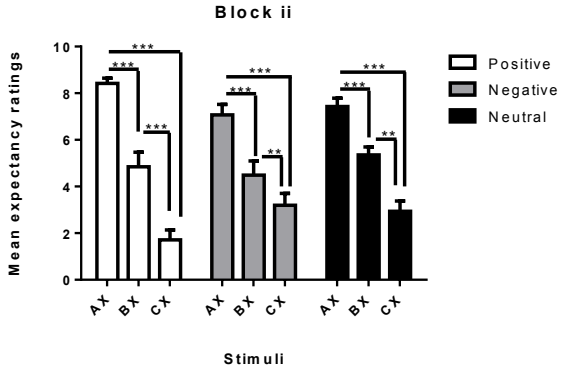
Expectancy ratings

Figures 3a/b/c show the expectancy ratings for AX, BX and CX trials during the Pavlovian learning for each condition. To simplify reporting of the results the block

variable was collapsed so that blocks 1 and 2 were collapsed (to create block i – figure 3(a)), blocks 3 and 4 were collapsed (to create block ii – figure 3(b)), and 5 and 6 collapsed into block iii (figure 3(c)) to match the learning pattern observed over time in figure 2 such that block i represented learning for the positive condition, block ii for the neutral condition, and block iii for the negative; this allowed for the data to be analysed more clearly. There was a significant main effect of trial type ($F(2, 54) = 141.51$, $p<.001$), this effect was determined by the pre-selection of participants, on the basis that they showed significant veridical knowledge over a minimum of thirty consecutive trials. A trial type by condition interaction ($F(4, 54) = 2.77$, $p=.036$) was also observed, as well as a block (time) by trial type interaction ($F(4, 108) = 18.65$, $p<.001$) and block (time) by trial type by condition interaction ($F(8, 108) = 2.12$, $p=.04$). Further analysis revealed that within block i those in the positive condition had acquired the greatest contingency knowledge such that ratings for all stimuli were different from each other ($p<0.001$), this degree of separation was not observed in the negative or neutral groups until block iii. Additionally, in block i those in the negative condition had yet to separate AX > BX ($p>0.05$). Furthermore, those in the negative condition rated CX higher than those in the positive condition in block i ($p<0.05$) and block ii ($p<0.05$), and in block i also rated AX lower than those in the positive condition ($p<0.05$). These comparisons of each condition to each other indicated that differential separation of expectancies was present in each condition despite the Pavlovian contingencies being the same, and may indicate a reduction in the speed of learning in the negative condition, perhaps driven by a lack in motivation, or perhaps represent a reduction in confidence to respond in a way that would demonstrate acquiring of contingency knowledge. It is worthwhile to note that for each condition the same percentage of participants, who completed the experiment, ultimately became aware.



(a)



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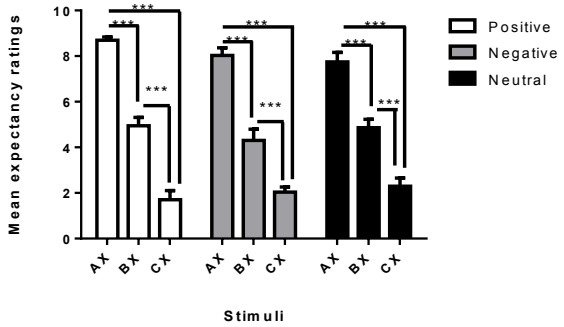
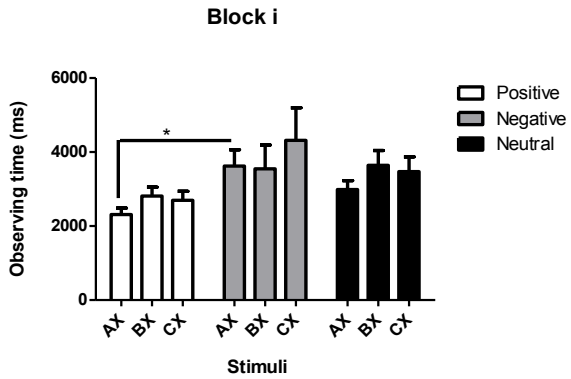


(c)

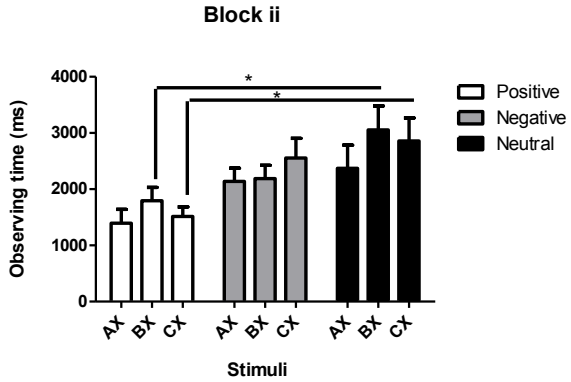
Figure 3: *Mean expectancy ratings following the presentation of trials AX, BX, CX during Pavlovian training in block i (a) block ii (b) and block iii (c); * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Block iii demonstrates discrimination occurring in aware participants across all conditions, not fully manifested in block i or ii by those participants in the negative and neutral conditions.*

Observing time

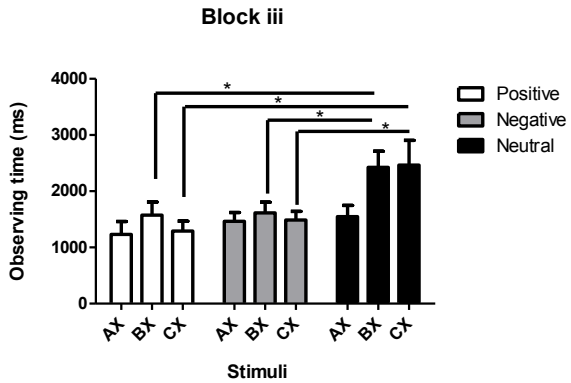
The block variable was collapsed as above to create the time (collapsed blocks) variable with 3 levels (blocks i, ii and iii). Figures 4a, b and c show that observing times during Pavlovian training should a trend to be greater for the uncertain BX trials than certain predictor and non-predictor trials, AX and CX, once the contingency knowledge had become acquired which is consistent with the results of Hogarth, Dickinson, et al. (2008), and further demonstrates that observing time can be used to index the predictive uncertainty of stimuli. In the present study there was a significant main effect of time ($F(1.31, 35.27) = 40.86$, $p<.001$) and trial type ($F(2, 54) = 9.16$, $p<.001$). A trial type by condition interaction also approached significance ($F(4, 54) = 2.50$, $p=.053$), such that observing time was lower in the positive condition than neutral, in blocks i and ii for both BX ($p<.05$) and CX ($p<.05$) trials. The same effect was seen in the negative condition when compared with the neutral, but limited to block iii ($p<.05$).



(a)



(b)



(c)

Figure 4: *Mean observing times (ms) for trials AX, BX, CX during the Pavlovian training in block i (a) block ii (b) and block iii (c); * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

Dwell time

Figure 5 shows percentage dwell times scores for the informative (A, B, C) and uninformative X stimuli (XA – when paired with stimulus A, XB when paired with stimulus B and XC when paired with stimulus C) during Pavlovian training block 6 (once all aware participants had successfully acquired the outcome contingencies). Analysis was performed using a 6 (stimuli – A/B/C/XA/XB/XC) by 3 (condition – positive/negative/neutral) ANOVA design. There was a significant main effect of stimulus ($F(2.84, 76.77) = 5.50$, $p=.002$) for the aware group (on investigating this effect values greater than two times the standard deviation of the mean were excluded), where stimulus A was selected from the contextual stimulus X (in XA trials) to a greater extent than stimulus B (in XB trials) or C (in XC trials) in the positive ($A>XA$, $p<.05$) and neutral ($A>XA$, $p<.01$) conditions; this effect on gaze preference for the informative stimuli in A trials was abolished in the negative condition such that attention apportioned to A was not greater than to XA ($p>.05$). There was, however, no difference in percentage dwell time between informative Stimuli A, B and C within conditions. These results are partially inconsistent with Hogarth, Dickinson, et al. (2008) and the discrepancies are difficult to account for. The unaware group showed no significant effects or interactions.

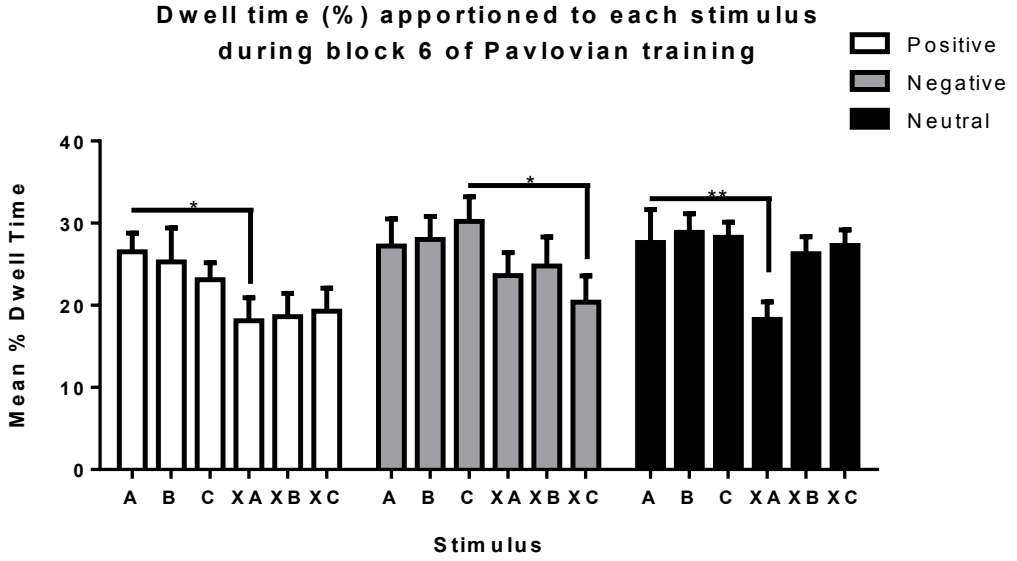


Figure 5: *Mean dwell time as a percentage of total observing time for stimulus A, B, C and uninformative X stimulus (XA – when paired with stimulus A, XB when paired with stimulus B and XC when paired with stimulus C) during block 6 of the Pavlovian training schedule for Negative, Neutral and Positive conditions. Error bars represent SE of mean. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

Pupil size

Figure 6 shows the average pupil size to informative stimuli by block and split by condition. There was a significant main effect of block ($F(2.45, 66.20) = 15.40$, $p<.005$) and a block by condition interaction ($F(4.90, 66.20) = 2.45$, $p = .043$), such that pupil size in the positive condition was greater than in the negative condition or neutral condition.

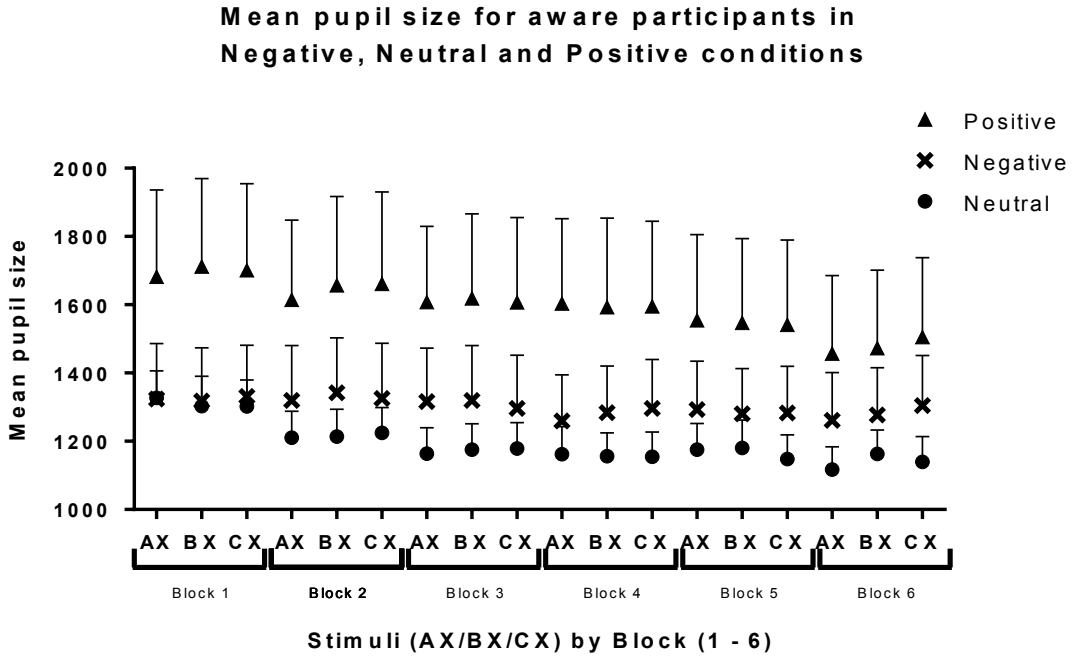
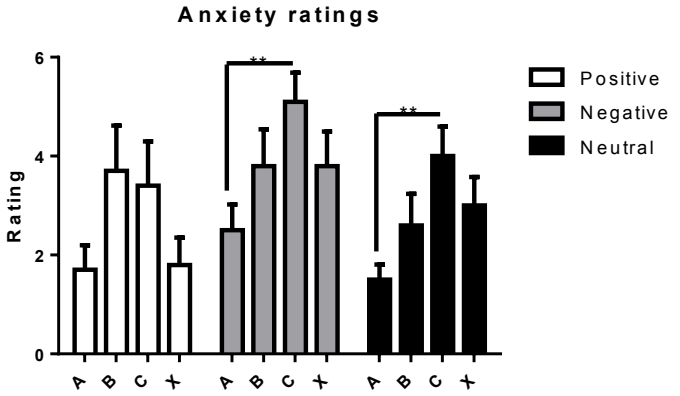


Figure 6: *Mean pupil size (μm) for each informative stimulus – A, B and C. Error bars represent SE of mean.*

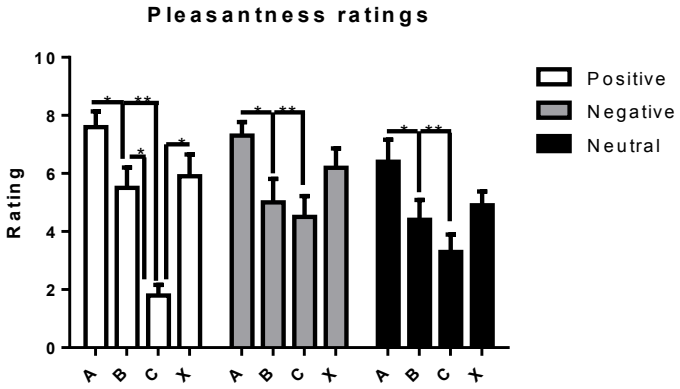
Evaluative conditioning

Figure 7(a) shows the anxiety ratings to stimuli reported at the end of the task. For aware participants there was a significant main effect of stimulus ($F(3, 81) = 7.45$, $p<.001$), such that stimulus A produced lower ratings of anxiety than stimulus C ($p<.01$) in the Neutral Mood group; this was maintained in the Negative condition, but abolished in the Positive condition. This main effect of stimulus was not observed in unaware participants ($F(3, 48) = 0.36$, $p=0.779$). There was also a significant effect of stimulus on ratings of pleasantness in aware (data shown in figure 7(b)) participants ($F(3, 81) = 21.24$, $p<.001$.), but not in the unaware group ($F(3, 48) = 0.92$, $p=0.438$). For aware participants, across all conditions, stimulus A produced higher ratings of pleasantness than B ($p<.05$) and also higher ratings that stimulus C ($p<.01$), and a trend

of B > C was also observed. This trend of stimulus B > C was significant in the Positive mood condition ($p<.05$). Furthermore, interestingly, the rating of pleasantness for stimulus C was significantly lower in the Positive condition compared to the Negative condition ($p<.01$).



(a)



(b)

Figure 7: *Mean emotional ratings for each stimulus - A, B, C and for the common stimulus X in the compound. Error bars represent SE of mean. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.*

Discussion

The effects of negative mood on learning processes have been extensively investigated (e.g. Clark, et al., 2005; Cools, Robinson, et al., 2008; Robinson, et al., 2009; Robinson & Sahakian, 2009b, etc) and more specifically the effect of mood on motivation to process reward cues (Rogers, et al., 2003; Roiser, et al., 2006) and on learning associations between stimuli and rewards (Chase, et al., 2010). The present study aimed to investigate further the effects of mood on associative learning by comparing directly positive and negative mood. Furthermore the present study examined how mood affects attention allocation and emotional reactivity to conditioned stimuli. As predicted, participants in the negative mood condition took longer to successfully become aware of the Pavlovian contingencies, and a reduced discrimination between expected gains has also been demonstrated previously (Rogers, et al., 2003) which may account for the delayed learning in the negative mood condition in the present study.

The prediction that attention to the stimuli would accord with the predictive uncertainty such that observing times would be greatest for the partially predictive compound BX compared to the reliable compounds AX and CX was not fulfilled. Furthermore, we hypothesised that the same pattern would be found in the dwell time data obtained with the eye tracker, such that the predictive stimuli in the compound would be gazed at for longer than the common X stimulus in BX trials, but not in AX or CX trials. However, contrary to our prediction, it was found that the predictive stimuli in the compound was gazed at for longer than the common X stimulus in AX trials, albeit only in the neutral and positive mood conditions. This effect was abolished in the negative mood condition, and replaced by a gaze preference towards the non-predictive stimulus C, over the concurrently presented X stimulus, in CX trials. This indicates a bias to the appetitive stimulus in the positive and neutral mood conditions but a bias for the

aversive stimulus (stimulus C - which may be viewed as a punishment stimulus) in the negative mood condition, supporting previous data associating negative mood with increased punishment prediction but not reward prediction (Cools, Robinson, et al., 2008; Rogers, et al., 2003).

The finding that those under state negative mood took longer to acquire the contingency knowledge correlates with previous findings that those in a state of negative mood show reduced learning (Chase, et al., 2010). It is worth to note, that their data obtained with the state negative mood group were confounded by a high BDI score within this group, but this was not the case in our study whereby the state mood conditions were matched for the BDI scores. Although a suppressed motivation for learning is the most commonly proposed explanation for reduced learning under state negative mood, in our study it is possible that reduced learning might have also reflected decreased confidence. This is likely as our study required the participants to make explicit responses indicative of learning, thus the reduced speed of learning may be driven also by a reduction in confidence to provide the correct response. It is known that confidence to produce explicit ratings in related measures are found to be decreased in negative mood conditions (Allan, et al., 2007). The reduced motivation account is more likely to be associated with the delayed learning in the negative mood condition found here, as negative mood and reduced motivation have long been linked (Cools, et al., 2005). A reason for the reduced motivation in negative mood may be associated with the perceived value of the reward being altered due to a state of anhedonia. However, in the present study the emotional responses to the stimuli (A, B, C and X) in the negative mood condition did not differ from the neutral mood condition as the ratings of pleasantness in both groups were higher for the A stimulus than for the C stimulus.

An interesting finding in the present study was the presence of an increased pupil size in the positive mood condition throughout the associative learning phase when compared to the negative and neutral mood conditions. It seems that positive mood creates a strong emotional state as links between pupil size and emotion has been recently reported (Kashihara, Okanoya, & Kawai, 2013; Kret, Roelofs, Stekelenburg, & de Gelder, 2013; Naber, Frassle, Rutishauser, & Einhauser, 2013; Prehn et al., 2013). Furthermore recent research has proposed that motivation can act as a driver for visual attention and has introduced pupil size as a indirect measure of motivation/effort (Wykowska, et al., 2013). In accordance to this proposal a link between action control and attentional processes has been hypothesised (Bekkering & Neggers, 2002; Wykowska, et al., 2009). Indeed the increased pupil size was accompanied by an increased attentional preference for AX trials and may have also driven the faster learning in the positive mood group as those under positive mood also acquired contingency knowledge earlier in the current study, albeit only in comparison to negative mood. These findings taken together also with our previous findings, (Mathers, Steckler, et al., 2014) demonstrate a reduced motivation to perform an instrumental response in the presence of predictive stimuli to gain reward or avoid punishment despite showing awareness of the relevant outcome when under induced state negative mood. Hence the present data support the suggestion that those under state negative mood are less motivated than those under positive mood to perform an action.

In summary, the present study reported that participants who underwent negative mood induction took longer to acquire Pavlovian contingency knowledge than those in either neutral or positive mood condition. Induced mood state also disrupted observation patterns to the stimuli such that induced mood reduced overall observation time in BX and CX trials, as well those in a negative mood state only showing a increased bias for

stimulus C over X. Therefore, positive and negative state mood differentially affect emotional and observational responses to Pavlovian stimuli predictive of reward, and also impact on the rate of acquiring contingency knowledge of the same predictive stimuli.

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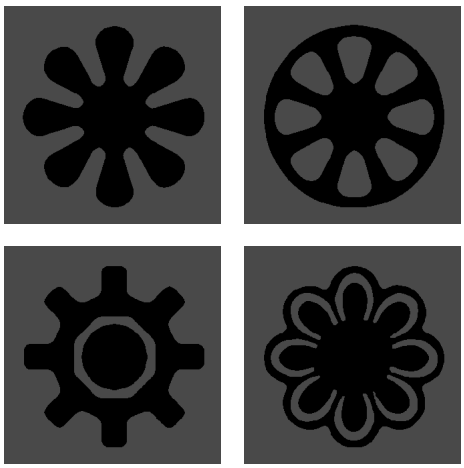
Appendix

Appendix 1: Grey-scale visual stimuli used for conditioned stimuli



Appendix 2: Profile of Mood States (POMS) questionnaire

Please rate from 0= not at all to 4=extremely, how the different adjectives represent your current mood state

Not at all	A little	Moderately	Quite a bit	Extremely		Not at all	A little	Moderately	Quite a bit	Extremely	
0	1	2	3	4	Friendly	0	1	2	3	4	Lonely
0	1	2	3	4	Tense	0	1	2	3	4	Miserable
0	1	2	3	4	Happy	0	1	2	3	4	Efficient
0	1	2	3	4	Angry	0	1	2	3	4	Bitter
0	1	2	3	4	Worn out	0	1	2	3	4	Pleased
0	1	2	3	4	Unhappy	0	1	2	3	4	Alert
0	1	2	3	4	Confused	0	1	2	3	4	Ready to fight
0	1	2	3	4	Lively	0	1	2	3	4	Restless
0	1	2	3	4	Unable to concentrate	0	1	2	3	4	Good-natured
0	1	2	3	4	Sorry for things done	0	1	2	3	4	Gloomy
0	1	2	3	4	Shaky	0	1	2	3	4	Desperate
0	1	2	3	4	Listless	0	1	2	3	4	Rebellious
0	1	2	3	4	Overjoyed	0	1	2	3	4	Nervous
0	1	2	3	4	Peeved	0	1	2	3	4	Helpless
0	1	2	3	4	Agreeable	0	1	2	3	4	Weary
0	1	2	3	4	Sad	0	1	2	3	4	Elated
0	1	2	3	4	Active	0	1	2	3	4	Forgetful
0	1	2	3	4	On edge	0	1	2	3	4	Deceived
0	1	2	3	4	Grouchy	0	1	2	3	4	Full of pep
0	1	2	3	4	Fatigued	0	1	2	3	4	Warm-hearted
0	1	2	3	4	Muddled	0	1	2	3	4	Carefree
0	1	2	3	4	Blue	0	1	2	3	4	Furious
0	1	2	3	4	Energetic	0	1	2	3	4	Uncertain about things
0	1	2	3	4	Spiteful	0	1	2	3	4	Worthless
0	1	2	3	4	Hopeless	0	1	2	3	4	Anxious
0	1	2	3	4	Satisfied	0	1	2	3	4	Vigorous
0	1	2	3	4	Panicky	0	1	2	3	4	Terrified
0	1	2	3	4	Helpful	0	1	2	3	4	Good-tempered
0	1	2	3	4	Unworthy	0	1	2	3	4	Guilty
0	1	2	3	4	Annoyed	0	1	2	3	4	Bushed
						0	1	2	3	4	Bad-tempered
0	1	2	3	4	Cheerful	0	1	2	3	4	Refreshed
0	1	2	3	4	Exhausted						
0	1	2	3	4	Resentful						
0	1	2	3	4	Forgiving						
0	1	2	3	4	Discouraged						
0	1	2	3	4	Relaxed						
0	1	2	3	4	Bewildered						
0	1	2	3	4	Sluggish						
0	1	2	3	4	Uneasy						
0	1	2	3	4	Kindly						

Month ☐

Day ☐

Year ☐

Time ☐

Initials ☐

Appendix 3: Drug Use questionnaire

Substance	Ever used (y/n)	Duration of use (mths/yrs)	Time since last use (days – yrs)	How often used per (wk/mo/yr)	Usual dose per session
Marijuana					(joints)
Cannabis					
Hashish					
Stimulants:					(grams)
Cocaine					
Crack					
Speed					
Ecstasy					(tabs)
Hallucinogens:					(hits)
Mushrooms					
LSD					
PCP					
Mescaline					
Ketamine					
Opiates:					(grams)
Heroin					
Morphine					

Barbiturates	(tabs)
Downers -	
(state type)	
Benzodiazepines	(tabs)
Tranquilizers -	
(state type)	
Anti-depressants	(tabs)
(state type)	
Inhalants:	(hits)
Poppers	
Glue	
Other	
(please specify)	

Appendix 4: Barratt Impulsiveness Scale questionnaire

Please circle the number which corresponds to the choice that best describes you. Try to describe the way you USUALLY act and feel, not just how you are feeling right now.

1 = rarely/never 2 = occasionally 3 = often 4 = almost always/always

1	I plan tasks carefully.	1 2 3 4
2	I do things without thinking.	1 2 3 4
3	I make up my mind quickly.	1 2 3 4
4	I am happy-go-lucky.	1 2 3 4
5	I don't "pay attention".	1 2 3 4
6	I have "racing" thoughts.	1 2 3 4
7	I plan trips well ahead of time.	1 2 3 4
8	I am self-controlled.	1 2 3 4
9	I concentrate easily.	1 2 3 4
10	I save regularly.	1 2 3 4
11	I "squirm" at plays or lectures.	1 2 3 4
12	I am a careful thinker.	1 2 3 4
13	I plan for job security.	1 2 3 4
14	I say things without thinking.	1 2 3 4
15	I like to think about complex problems.	1 2 3 4
16	I change jobs.	1 2 3 4
17	I act "on impulse".	1 2 3 4
18	I get easily bored when solving thought problems.	1 2 3 4
19	I act on the spur of the moment.	1 2 3 4
20	I am a steady thinker.	1 2 3 4
21	I change residences.	1 2 3 4
22	I buy things on impulse.	1 2 3 4
23	I can only think about one problem at a time.	1 2 3 4
24	I change hobbies.	1 2 3 4
25	I spend or charge more than I earn.	1 2 3 4
26	I often have extraneous thoughts when thinking.	1 2 3 4
27	I am more interested in the present than the future.	1 2 3 4
28	I am restless at the theatre or lectures.	1 2 3 4
29	I like puzzles.	1 2 3 4
30	I am future orientated.	1 2 3 4

Appendix 5: Beck Depression Inventory (BDI) questionnaire

On this questionnaire are groups of statements. Please read each group of statements carefully. Then pick out the *one* statement which best describes the way you have been feeling **for the past week, including today.** Circle the number or underline the statement you choose. Be sure to read all the statements in each group before making your choice.

1.
0 I do not feel sad.
1 I feel sad.
2 I am sad all the time and I can't snap out of it.
3 I am so sad and unhappy that I can't stand it.

2.
0 I am not particularly discouraged about the future.
1 I feel discouraged about the future.
2 I feel I have nothing to look forward to.
3 I feel the future is hopeless and that things cannot improve.

3.
0 I do not feel like a failure.
1 I feel I have failed more than the average person.
2 As I look back on my life, all I can see is a lot of failures.
3 I feel I am a complete failure as a person.

4.
0 I get as much satisfaction out of things as I used to.
1 I don't enjoy things the way I used to.
2 I don't get real satisfaction out of anything anymore.
3 I am dissatisfied or bored with everything.

5.
0 I don't feel particularly guilty.
1 I feel guilty a good part of the time.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6.
0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7.

0 I don't feel disappointed in myself.
1 I am disappointed in myself.
2 I am disgusted with myself.
3 I hate myself.

8.

0 I don't feel I am any worse than anybody else.
1 I am critical of myself for my weaknesses or mistakes.
2 I blame myself all the time for my faults.
3 I blame myself for everything bad that happens.

9.

0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10.

0 I don't cry any more than usual.
1 I cry more now than I used to.
2 I cry all the time now.
3 I used to be able to cry, but now I can't cry even though I want to.

11.

0 I am no more irritated by things than I ever was.
1 I am slightly more irritated now than usual.
2 I am quite annoyed or irritated a good deal of the time.
3 I feel irritated all the time.

12.

0 I have not lost interest in other people.
1 I am less interested in other people than I used to be.
2 I have lost most of my interest in other people.
3 I have lost all of my interest in other people.

13.

0 I make decisions about as well as I ever could.
1 I put off making decisions more than I used to.
2 I have greater difficulty in making decisions more than I used to.
3 I can't make decisions at all anymore.

14.

0 I don't feel that I look any worse than I used to.
1 I am worried that I am looking old or unattractive.
2 I feel that there are permanent changes in my appearance that make me look unattractive.
3 I believe that I look ugly.

15.

0 I can work about as well as before.
1 It takes an extra effort to get started at doing something.
2 I have to push myself very hard to do anything.
3 I can't do any work at all.

16.

0 I can sleep as well as usual.
1 I don't sleep as well as I used to.
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3 I wake up several hours earlier than I used to and cannot get back to sleep.

17.

0 I don't get more tired than usual.
1 I get tired more easily than I used to.
2 I get tired from doing almost anything.
3 I am too tired to do anything.

18.

0 My appetite is no worse than usual.
1 My appetite is not as good as it used to be.
2 My appetite is much worse now.
3 I have no appetite at all anymore.

19.

0 I haven't lost much weight, if any, lately.
1 I have lost more than five pounds.
2 I have lost more than ten pounds.
3 I have lost more than fifteen pounds.

I am purposely trying to lose weight by eating less: YES____ NO____

20.

0 I am no more worried about my health than usual.
1 I am worried about physical problems such as aches and pains, or upset stomach, or constipation.
2 I am very worried about physical problems and it's hard to think of much else.
3 I am so worried about my physical problems that I cannot think about anything else.

21.

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I have almost no interest in sex.
3 I have lost interest in sex completely.

Appendix 6: Medical Health questionnaire

Nuffield Hospitals Medical History Questionnaire

Confidential

Please complete all sections of this form unless otherwise indicated.

Name (Full).....

Date of Birth..... Sex..... Height..... Weight.....

Please underline the appropriate answer where a **'Yes'** or **'No'** is required. If your answer is **'Yes'** brief details should be given.

1. Have you suffered from any of the following?

		Details
Diabetes Mellitus	**Yes / No**	
Epilepsy	**Yes / No**	

Frequent chest, throat or nose infections/diseases **Yes / No**

Back injury/backache **Yes / No**

Joint injury **Yes / No**

Ear infection **Yes / No**

Rheumatism or Rheumatic fever **Yes / No**

Urinary problems or kidney disease **Yes / No**

Infectious diseases (Mumps, Measles, German Measles, Tuberculosis etc.) **Yes / No**

Hepatitis **Yes / No**

Heart disease **Yes / No**

High blood pressure, chest pain,

shortage of breath **Yes / No**

Anxiety or Depression requiring treatment **Yes / No**

Nervous breakdown or debility arising

from overwork **Yes / No**

Menstrual problems **Yes / No**

Haemorrhoids **Yes / No**

Dyspepsia or Peptic Ulcer **Yes / No**

Hernia **Yes / No**

Dysentry/Typhoid/Food poisoning **Yes / No**

Any other stomach disorder **Yes / No**

Varicose veins **Yes / No**

Migraines or other frequent headaches **Yes / No**

Hay fever, eczema or other allergies **Yes / No**

Skin disorders **Yes / No**

Fainting or giddiness **Yes / No**

Poor eyesight (even when wearing glasses/contact lenses) **Yes / No**

Please give date when eyesight was last tested (approx.) **Yes / No**

Impaired hearing **Yes / No**

2. Are you a registered disabled person? **Yes / No** If **'Yes'** what is you registration number and expiry date?

_____

_

3. a) Have you been an in-patient in hospital or consulted your GP during the last five years? **Yes / No** If **'Yes'** please give details:

b) How many days of sickness have you had in the last 12 months? What were the main causes?

c) Are you taking any pills, tablets or having injections, receiving any medical or psychiatric treatment or advice or awaiting surgery? **Yes / No** If **'Yes'** please give details:

_____

_

4. How often do you visit your dentist? When was your last visit?

_____

5. What was the date of your last Tetanus

immunisation against the following:

Tuberculosis

Polio

Rubella (German Measles)

(Anti-D Gammaglobulin)

Hepatitis B

_____

_

6. Date of last x-ray Reason for x-ray

_____

_

7. General state of health; please comment on any aspects not covered above (i.e. accidents, injuries, disorders not mentioned).

8. What is your average consumption of a) alcohol units* per week

(* A unit- single measure of spirit /one glass of wine/ half a pint of beer)

b) tobacco per day

9. Is there any additional information regarding your health not covered in the above questions?

I declare that the answers given to the above questions are true to the best of my knowledge and I have not withheld any material facts which may have any bearing as to the state of my health.

Signature Date

Appendix 7: Visual Analogue Scales (VAS)

Subjective mood ratings

How do you feel **NOW**? Please draw a vertical mark on each line, in the position you feel best represents your current state.

Sad

not at all _____ very much

Contented

not at all _____ very much

Stimulated

not at all _____ very much

Happy

not at all _____ very much

Relaxed

not at all _____ very much

Appendix 8: Table 1 – Summary of studies investigating the effects of mood

Citation	Participants	Intervention	Measures	Results
(Rogers, et al., 2003)	N = 18 (healthy volunteers)	ATD vs. placebo	- decision making (gambling) task	ATD altered decision making by reducing discrimination between magnitudes of expected gains
(Cools, et al., 2005)	N = 10 (within subject) N = 23 (between subject) (healthy volunteers)	ATD vs. placebo	- VAS - CRRT - Stop-signal reaction-time task	ATD slowed responses in the CRRT increasing accuracy whilst showed no effects on the ability to inhibit responses or on mood.
(O. Robinson, et al., 2009)	N = 11 (healthy volunteers)	ATD vs. placebo (Negative vs. positive vs. neutral mood)	- CRRT - SRET	Mood state moderates the role serotonin in cognitive biases.

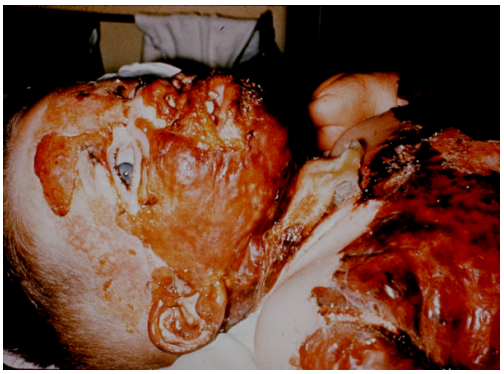
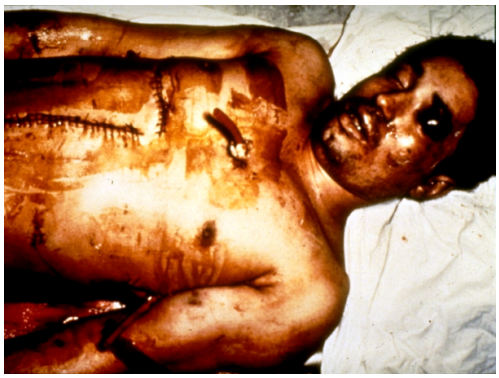
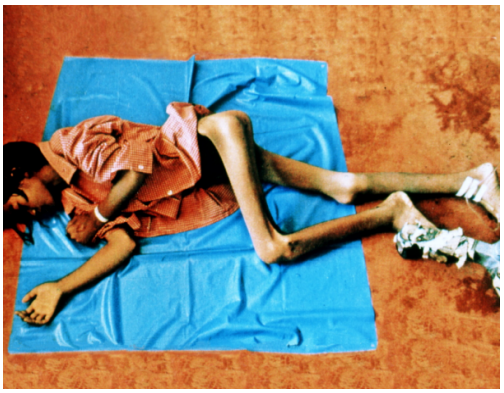
(O. J. Robinson & Sahakian, 2009b)	N = 11 (healthy volunteers)	ATD vs. placebo (Negative vs. positive vs. neutral mood)	- AGNG - OTT	Mood induction affected "cold" (OTT) tasks through a top-down mechanism, while ATD affected "hot" (AGNG) tasks through a bottom-up process.
(Jans, et al., 2008)	N = 24 (12 male SD rats and 12 male BN rats – 3 months of age)	ATD vs. placebo (vehicle)	- anxiety related behaviour tests (open-field test, home cage emergence test and social interaction test) - depression related behaviour test (forced swim test) - cognition test	SD rats showed increased depression and anxiety after ATD; BN rats did not. ATD reduced plasma serotonin in BN and SD rats but reduced hippocampal serotonin in BN rats only. ATD effects are strain dependent.

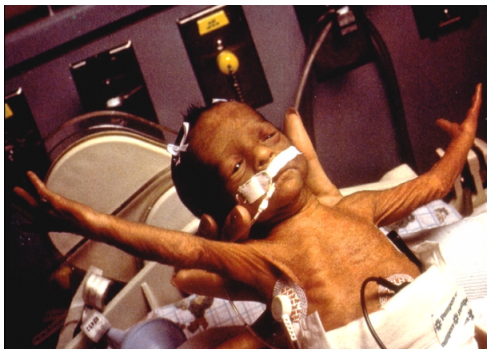
			(object recognition test)	
(Clark, et al., 2005)	N = 41 (Healthy volunteers; 19 short-short transporter alleles, 15 long-long, 7 short-long)	ATD vs. placebo	- trait impulsivity (Barratt Impulsivity Scale) - SST	No effect of ATD against placebo was observed on the SST.
(Finger, et al., 2007)	N = 16 (Healthy volunteers)	ATD vs. placebo	- 5-HTTLPR genotyping - Passive avoidance learning task - Probabilistic response reversal task	Genotype plays a role in the effects of ATD observed during certain cognitive and emotional tasks.
(Blair, et al., 2008)	N = 24 (Healthy volunteers)	ATD vs. placebo ATD (N=11): 7 female, 8	- Differential reward/ punishment task	ATD and long-long homogenisity induced reduced sensitivity to punishment-based

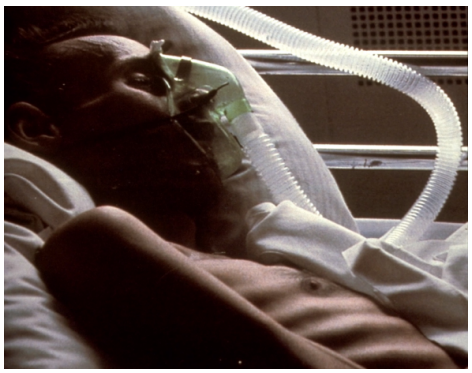
		short allele carriers. Placebo (N=13): 6 female, 7 short carriers.		information.

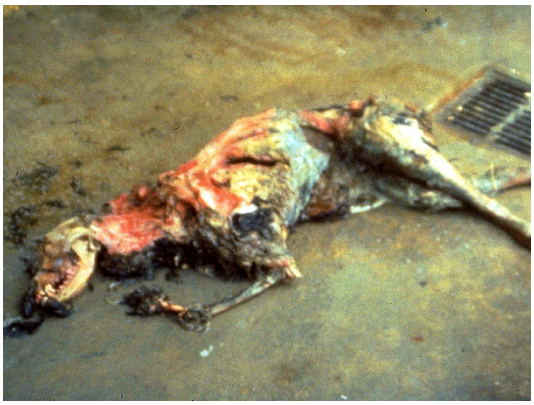
ATD; acute tryptophan depletion. VAS; visual analogue scale. CRRT; cued-reinforcement reaction-time task. SRET; self-referent encoding/retrieval task. AGNG; affective go/no-go. OTT; one touch tower of london. SD; Sprague Dawley. BN; Brown Norway. SST; stop signal task. 5-HTTLPR; serotonin transporter

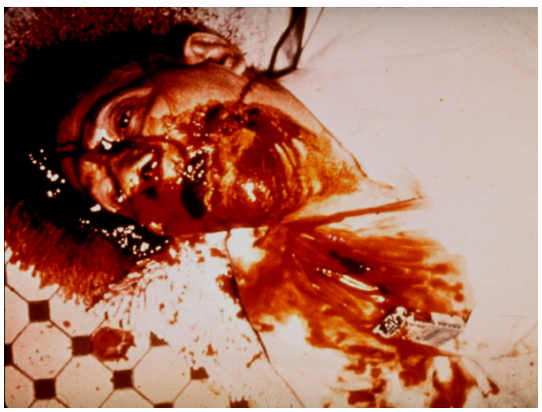
Appendix 9: Mood Induction Procedure Images - Negative mood condition

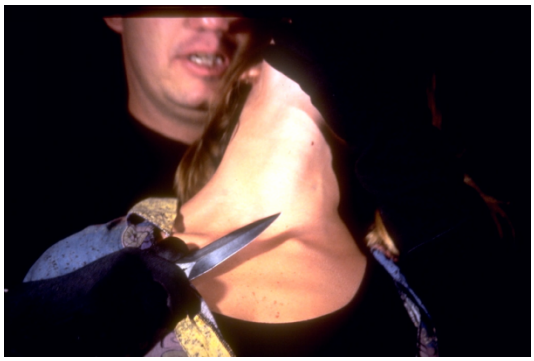




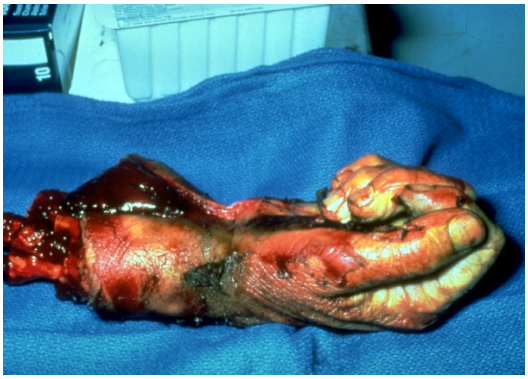



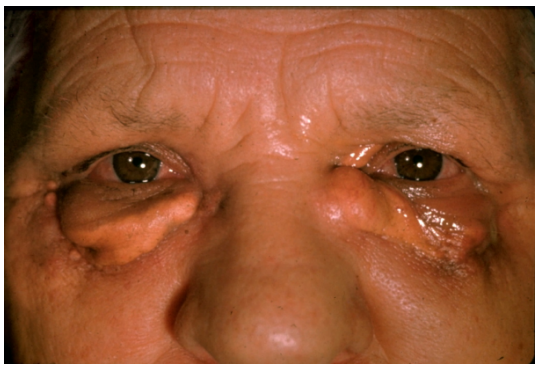


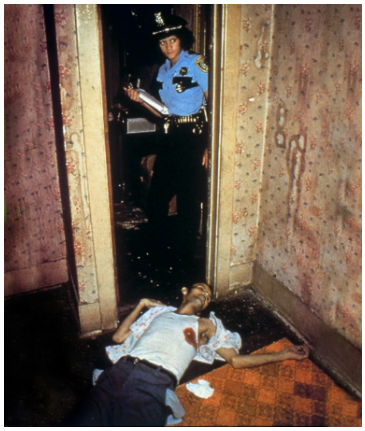


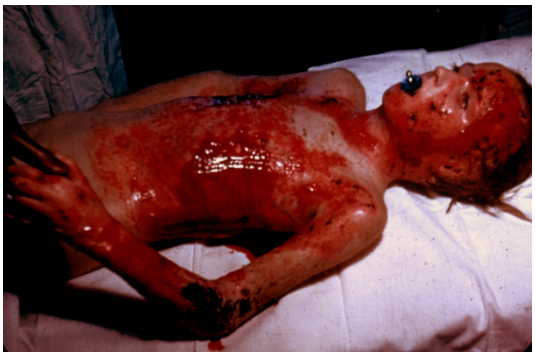


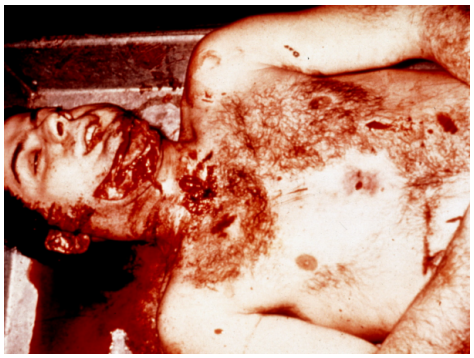


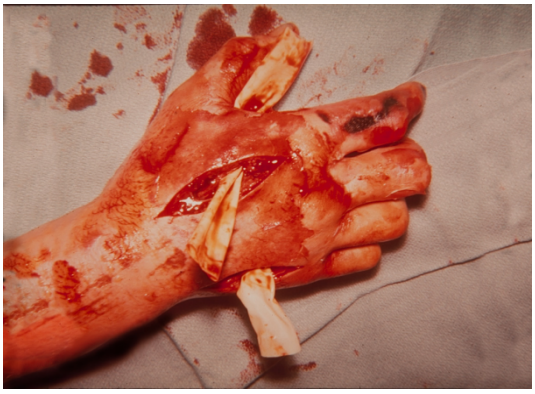


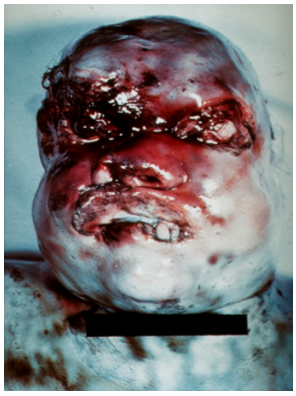




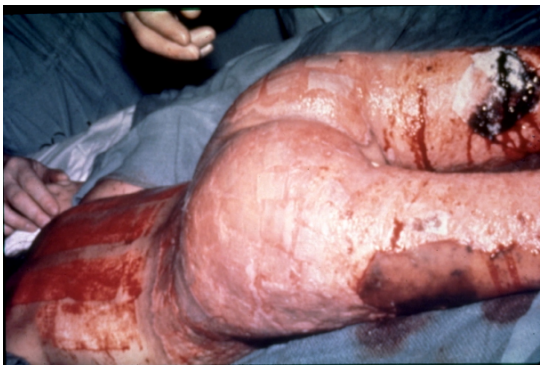


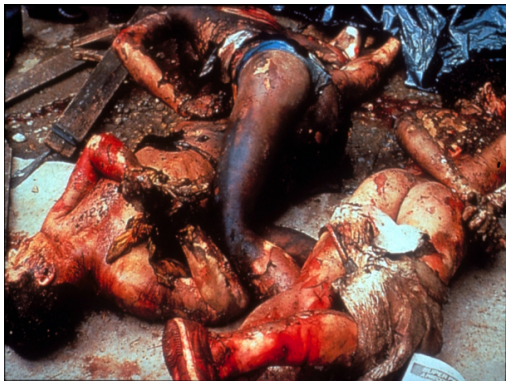


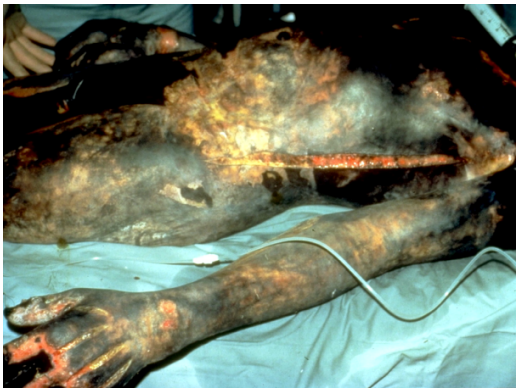


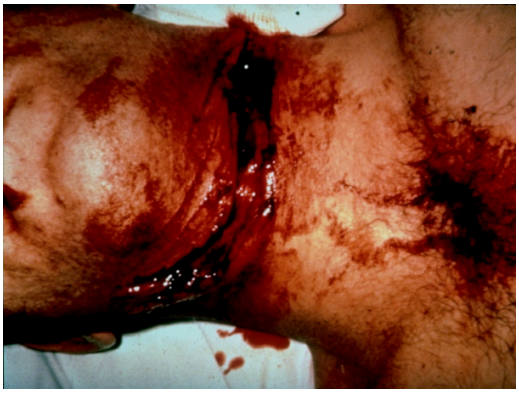



Blut Ehre







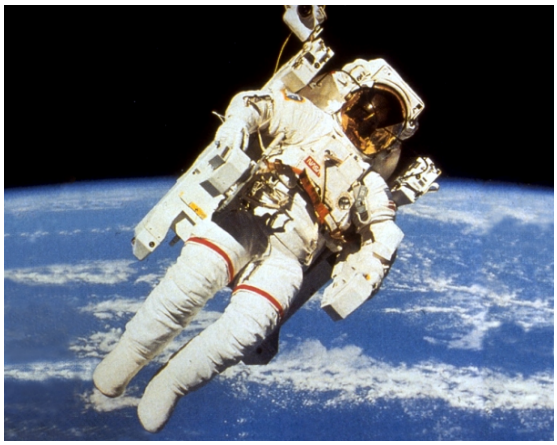


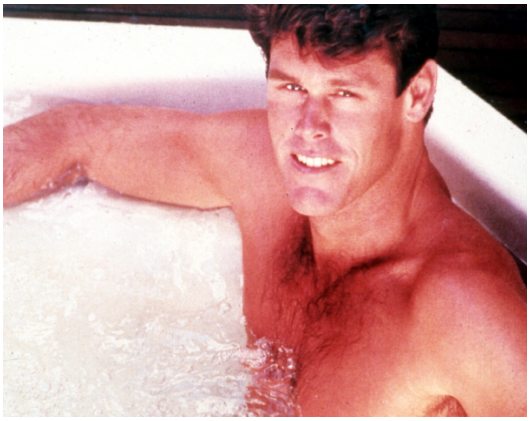
Appendix 10: Mood Induction Procedure Images - Positive mood condition: set 1

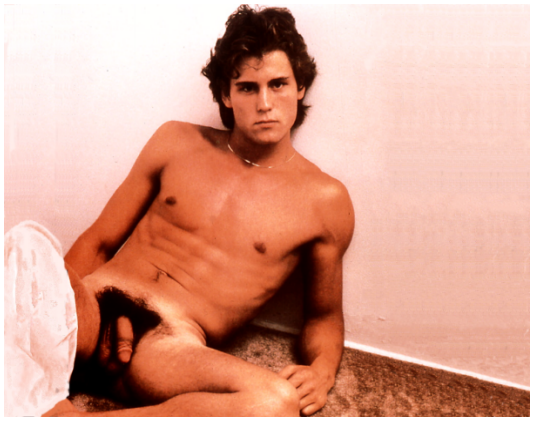


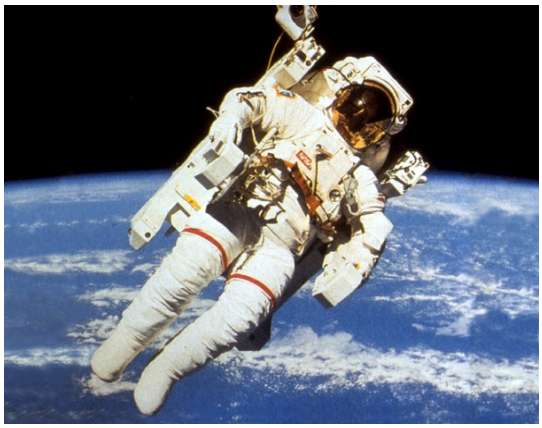










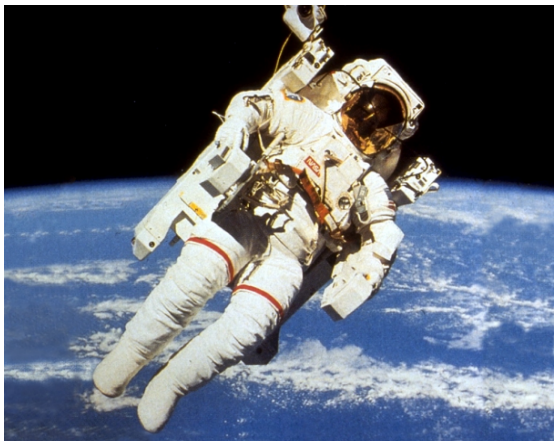

Hotel
Fremont
Golden
Nugget
MINT
Coin
Castle
FREE-FREE

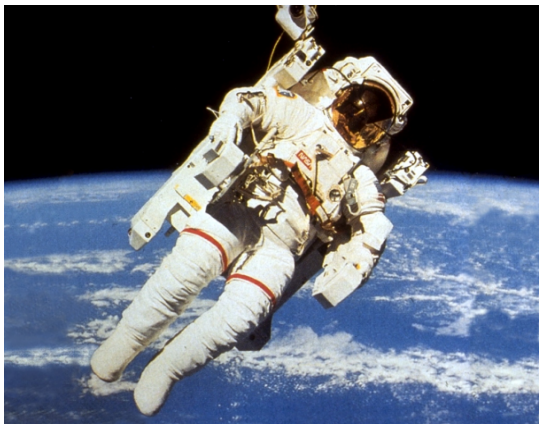
Appendix 11: Mood Induction Procedure Images - Positive mood condition: set 2










Hotel
Fremont
MINT
Golden
Nugget
Coin
Castle

Appendix 12: Mood Induction Procedure Images - Neutral mood condition