Supplementary Table 1- Contrast between Attend CS+ and CS– trials

Attend CS+ > Attend CS–

Region of Activation	*Laterality*	*Talairach Coordinates*			*Voxels*	*t-stat*
		x	*y*	*z*		
Cingulate Gyrus (BA 32)	R	4	28	38	841	3.75
Striatum (caudate nucleus)	R	8	6	6	514	3.68
Striatum (caudate nucleus)	L	-9	2	4	992	3.33
Insula	L	-31	17	0	1335	4.19
Insula	R	34	16	0	1755	4.54
Basal Forebrain	L	-9	-2	0	936	4.01
Basal Forebrain	R	11	-6	-2	626	3.29
Midbrain	L	-1	-26	-9	395	4.22
Midbrain	R	2	-15	-10	633	3.68

Attend CS– > Attend CS+

Region of Activation	*Laterality*	*Talairach Coordinates*			*Voxels*	*t-stat*
		x	*y*	*z*		
Precuneus (23/31)	R	6	-61	19	338	3.66

Supplementary Table 2- Contrast between Regulate and Attend trials

Regulate > Attend

Region of Activation	*Laterality*	*Talairach Coordinates*			*Voxels*	*t-stat*
		x	*y*	*z*		
Middle Frontal Gyrus (BA6/9)	L	-47	3	37	103	3.59
Postcentral Gyrus (BA1,2,3)	L	-46	-31	37	151	3.89
Inferior Parietal Lobule (BA40)	L	-37	-43	38	276	3.64
Superior Occipital Gyrus (BA19)	L	-32	-80	33	379	3.57
Inferior Frontal Gyrus (BA6/44)	L	-45	0	32	40	3.74
SubGenual (BA 25)	L	-3	12	-5	69	3.57

Attend > Regulate

Region of Activation	*Laterality*	*Talairach Coordinates*			*Voxels*	*t-stat*
		x	*y*	*z*		
Medial Frontal Gyrus (BA6)	L	-4	-4	67	239	3.64
Superior Frontal Gyrus (BA9)	R	3	50	38	184	3.71
Cuneus (BA19)	L	-5	-86	26	345	3.76
Thalamus		0	-13	13	651	3.88
Caudate Nucleus	L	-14	22	11	145	3.71
Insula	L	-44	-3	8	1148	4.13
Fusiform Gyrus (BA18)	R	20	-87	5	300	3.83
Cerebellum	R	26	-63	-23	377	3.78
Lingual Gyrus (BA18)	R	12	-74	-17	1036	3.81

Regulating the expectation of reward via cognitive strategies

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Supplementary Online Materials

Methods

Participants

A total of 22 participants were recruited using posted advertisements and gave written consent. From this initial group, 7 participants were excluded from further analysis due to a lack of physiological evidence of conditioning, as indicated by skin conductance responses. Thus, final analysis was conducted on 15 right-handed volunteers (9 male, 6 female; average age, *M*= 20.6, SD= 2.23).

Procedure

The experiment involved an appetitive or reward conditioning paradigm modelled after a previous study [1]. The paradigm included two conditioned stimuli (CS), either a blue or yellow square, that were either paired (CS+) or not paired (CS–) with a potential unconditioned stimulus (US), a monetary reward of $4.00. Participants were explicitly told the contingencies. Prior to CS presentation, participants were presented with a cue, a single word instruction that told participants to either Attend or Regulate the stimulus. The instruction was presented on a black background for 2 seconds, followed by the CS presentation for 4 seconds and an ISI of 12 seconds, during which time the participant saw a fixation point. The probabilistic US was

presented for 500ms and co-terminated with the CS+. Participants were instructed that they won $4.00 with each US presentation and accumulated the money throughout the experiment. Conditions were counterbalanced across participants. There were a total of 72 trials separated into 3 blocks of 24 trials each. Within these blocks, there were 15 trials per type of condition (Attend CS+, Attend CS–, Regulate CS+, Regulate CS–) and an additional 12 trials where the US was delivered (CS–US trials, 6 each for Attend and Regulate conditions). At the end of the study, participants' compensation was rounded to $60.

Instructions were adapted from a previous emotion regulation study [2], and involved imagery related strategies given the nature of the stimulus (i.e., colored square x detailed pictures). When the Attend instruction preceded a CS+ trial, the participant was instructed to think about the possibility of winning $4.00; if Attend was paired with the CS–, however, the participant was instructed to think about the fact that no money was gained. In contrast, when the instruction Regulate appeared on screen, participants were instructed to conjure a soothing image of a soothing image from nature prompted by the color of the square. For example, upon seeing a blue square, participant could imagine the ocean or blue sky, while imagining a sunny beach or a field of flowers for the yellow square. Participants were asked to generate the same image every time each color square was presented. In addition, participants were notified that regardless of the instruction, the CS+ always indicated the possibility of winning $4.00.

Before entering the scanner participants completed a set of practice trials and were asked to verbalize what they were thinking about during each condition to assure that they were following the instructions they were given. Prior to the scanning session, therefore, participants were aware of the contingencies (e.g., blue square predicted a potential monetary gain) and well-practiced in the instructions. At the end of each fMRI session, participants completed a survey to rate their subjective arousal during the different conditions of the task. Following each session participants were debriefed and given their payment.

Although the instructions of this experiment were adapted from a previous emotion regulation study [2], the instructions are different than most traditional regulation studies. Such studies generally vary in terms of paradigm and instructions, taking advantage of complex stimuli, such as ambiguous emotional pictures while often measuring emotion via subjective reports (See [3] for review). These studies typically use a "reappraisal" strategy that requires participants to reinterpret the meaning of a stimulus, such as a complex emotional picture, which may chance every trial. Instead, the current study utilizes repeated stimuli combined with an imagery instruction which remains constant across conditioned stimuli (e.g., squares). The strategy employed in the current study is particularly useful for drawing comparisons with cognitive behavioral therapy (CBT), an advantage of these instructions. In some forms of CBT, for instance, patients learn and repeatedly utilize specific strategies, such as imagery, in response to specific cues [4]. It is important to note, however, that a disadvantage of these instructions is the difficulty that arises in disentangling the regulatory effects from general emotion regulation process or just mere distraction, which by itself can also alter emotional responses [3]. Although this potential interpretation cannot be discounted and necessitates further studies, the results presented in this paper are consistent with an emotion regulatory account, including subjective, physiological and neural responses to the expectation of reward. Future studies will be necessary to disambiguate the various components that contribute to successful emotion regulation.

Physiological Set-up and Assessment

Stimulus presentation was controlled by a PC with E-Prime software. An LCD projector displayed stimuli on a screen behind the scanner, and participants were able to see the screen in a mirror mounted on the head coil. Conditioning was measured using differential skin

conductance response (SCR) and was collected using a Biopac MP100 system and recorded using Acknowledge software.

Skin conductance responses were acquired from the participant's middle phalanges of the second and third fingers in the left hand using BIOPAC systems skin conductance module and shielded Ag-AgCl electrodes grounded through an RF filter panel. AcqKnowledge software was used to analyze SCR waveforms. The level of SCR response was assessed as the base to peak difference in the .5 to 4.5 second window following the onset of a CS, the blue or yellow square (see [5]). SCRs for each participant was converted to standardized *T* scores and averaged per participant, per condition [6]. Trials in which the CS+ was paired with $4.00 were separated into time of CS+ presentation and time of US presentation so only differential SCR response to the CS+ was included. A repeated measures ANOVA was conducted with type of trial (Attend, Regulate) and type of stimulus (CS+, CS–) as within-subjects factor to investigate the effects of emotion regulation during conditioned fear.

fMRI Acquisition & Analysis

A 3T Siemens Allegra head-only scanner and a Siemens standard head coil were used for data acquisition at NYU's Center for Brain Imaging. Anatomical images were acquired using a T1-weighted protocol (256 x 256 matrix, 176 1-mm sagittal slices) Functional images were collected in the same slices using a gradient echo EPI sequence (TR= 2000ms, TE= 20 ms, FOV=192, flip angle= 75°, bandwidth = 4340 Hz/px, echo spacing = 0.29 ms, voxel size 3 x 3 x 3). Scanning was divided into three runs corresponding to three blocks of stimulus presentation. Thirty-nine contiguous oblique-axial slices (3 x 3 x 3 mm voxels) parallel to the AC-PC line were obtained.

Analysis of imaging data was conducted using Brain Voyager software (Brain Innovation, Maastricht, The Netherlands). The data was initially corrected for motion (using a threshold of 2

mm or less), and slice scan time using sinc interpolation was applied. One imaging run from one participant was removed due to excessive movement in the scanner (more than a 5mm shift in movement). Further, spatial smoothing was performed using a three-dimensional gaussian filter (4-mm FWHM), along with voxel-wise linear detrending and high-pass filtering of frequencies (3 cycles per time course). Structural and functional data of each participant was then transformed to standard Talairach stereotaxic space [7].

Group data were analyzed using a random effects model to determine blood oxygenation dependent signal (BOLD) for task-specific events. There were four predictors of interest (Attend CS+, Attend CS–, Regulate CS+, Regulate CS–) and four predictors of no interest (Attend instruction cue, Regulate instruction cue, CS–US trials, US delivery). Trials where the US was delivered (CS–US trials) were excluded from analysis. To determine event-related BOLD signals in specific brain areas, a general linear model of the experiment was imposed on the group image which revealed contrast activations when comparing specific trial types. Statistical maps of interest were created using a threshold of $p < 0.005$ and a cluster threshold of 4 contiguous voxels. Statistical parametric maps were created based on two specific contrasts which allowed the selection of *a priori* Regions of interest (ROIs in the striatum and PFC, specifically dorsolateral and medial PFC). These contrasts included 1) Attend CS+/Attend CS– contrast, motivated by previous research on reward expectations and prediction [8-10] and 2) Regulate/Attend contrast, motivated by previous research on emotion regulation [2]. All other reported regions in the whole brain analysis are exploratory.

Results

Physiological Assessment of Appetitive Conditioning

Analysis of SCR data was conducted on 22 participants to assure successful conditioning, as determined by total amount of skin responses to CS+ presentations during the

Attend condition (M=14.73, SD=4.06). Participants with one standard deviation below the mean (less than half of total trials during this condition) were removed. Specifically, seven participants who did not meet this threshold for a conditioned physiological response were removed so that later imaging analysis (final N=15) would accurately measure emotion regulation of the conditioned response to reward.

Post-hoc t-tests were conducted after the repeated measures two-way ANOVA reported in the manuscript. These t-tests revealed a significantly greater differential SCR to CS+ (M = 0.41, SD = 0.25) versus the CS– (M = 0.17, SD = 0.13) in Attend trials [$t(14) = 4.52$, $p < 0.0001$], while no differences were observed between CS+ (M = 0.15, SD = 0.11) and CS– (M = 0.15, SD = 0.12) Regulate trials [$t(14) = 0.08$, $p = 0.94$]. Participants also showed a significant decrease in SCR in Regulate of the CS+ compared to Attend trials of the CS+ [$t(14) = -4.65$, $p < 0.0001$], while no differences were observed between Regulate vs. Attend CS– trials [$t(14) = 0.62$, $p = 0.55$]. Importantly, no differences were observed when comparing US responses between Attend and Regulate trials ($t(14) = 0.14$, $p = 0.89$), suggesting that participants were focused on the conditioned stimulus irrespective of instructions and not distracted or averting their gaze. Finally, one way ANOVAs showed no main effect of counterbalancing (i.e., color square assigned to the CS+) in either Attend [$F(1,14) = 1.629$, $p = 0.224$] or Regulate trials [$F(1,14) = .256$, $p = 0.622$].

Subjective Ratings:

Subjective ratings acquired post-session, based on a post-experimental 7 point Likert scale, indicated that participants were significantly more excited by the opportunity to win money during Attend (M = 6.00, SD = 1.13) compared to Regulate (M = 4.07, SD = 1.39) trials ($t(14)= 3.42$, $p< 0.005$). They were also more excited about the chance of winning money in general (M = 5.53, SD = 1.06) than excited during Regulation trials ($t(14) = 3.37$, $p < 0.005$). In contrast, there were no differences between the excitement of winning money in general compared to

Attend trials ($t(14) = 1.7$, p = n.s.), suggesting similar subjective feelings with respect to winning in the Attend condition and winning overall, with both being higher than regulation. Further, there were no significant differences between the perceived difficulty of emotion regulation of the CS+ (M = 2.73, SD = 1.83) versus CS– (M = 2.33, SD =1.29) trials ($t(14) = 0.823$, p = 0.424), or the subjective feeling of ability to successfully regulate the CS+ (M=2.933, SD=1.44) or CS– (M=2.33, SD=1.11) trials ($t(14) = 1.60$, p = 0.132). These results support the physiological data and suggest that emotion regulation effectively decreased positive arousal elicited by the conditioned stimulus, further suggesting that emotion regulation strategies were similarly executed across types of conditioned stimuli (CS+ and CS–).

Neuroimaging Results

Attend CS+ vs. Attend CS– contrast

The primary contrast of interest was based on studies of reward expectation and prediction [8-10]. We hypothesized that, during the Attend condition, striatum activity would be associated with a salient CS, that is, a CS that predicts a potential reward. Results from this contrast are reported in Supplementary Table 1. Mean beta weights extracted from all ROIs were then input into separate repeated measures ANOVAs with factors of type of conditioned stimuli and type of instruction.

In accordance to previous research, both left and right striatum, specifically the anterior head of the caudate nucleus, were recruited by this contrast. An interaction between type of conditioned stimuli and type of instruction in both left (F (1,14) = 16.70, $p < 0.001$) and right (F (1,14) = 8.97, $p < 0.01$) striatum ROIs was also observed. Additionally, *post-hoc* t-tests in the left striatum ROI showed a differential response between Attend and Regulate CS+ ($t(14)= 2.35$, $p< 0.05$), but not CS– ($t(14)= 1.42$, $p= 0.18$) trials. Similar results were observed in the right striatum between Attend and Regulate CS+ ($t(14)= 2.13$, $p= 0.05$), but not CS– ($t(14)= -0.98$, $p=$

0.35) trials. These results suggest that emotion regulation strategies effectively attenuated increases in BOLD response typically observed by reward predicting conditioned stimuli. Activation of left and right striatum was observed as predicted.

A significant interaction between type of conditioned stimuli and type of instruction was also observed in other ROIs. These include the left insula (F (1,14) = 7.45, $p < 0.02$), the left (F (1,14) = 31.52, $p < 0.0001$) and right (F (1,14) = 14.43, $p < 0.002$) midbrain, and the left (F (1,14) = 23.32, $p < 0.0001$) and right (F (1,14) = 33.09, $p < 0.0001$) basal forebrain. The insula has been previously implicated in conditioning experiments, particularly aversive learning [11], but more recently it has been linked with risky decision-making [12,13]. Its involvement in emotion regulation of reward expectation is a novel finding that may be relevant to drug addiction research (e.g., [14]). Given the voxel resolution and the group analysis, it is difficult to localize the midbrain ROIs to a specific subnuclei such as the ventral tegmentum area or substantia nigra. However, midbrain activation in this task is suggestive of dopaminergic involvement during presentation of a conditioned stimulus that predicts reward [15]. Finally, the basal forebrain has more recently been linked to the interaction of motivation and effort in humans [16], perhaps suggesting that in our paradigm, the basal forebrain is modulating the levels of arousal or motivation according to the levels of cognitive effort.

Regulate vs. Attend contrast

The second contrast yielded a variety of cortical regions previously implicated in emotion regulation[2,17] (see Supplementary Table 2). Such regions included the middle frontal gyrus (BA 6/9; Fig. 2B) and inferior frontal gyrus (BA 6/44) and inferior parietal cortex (BA 40). Notably, the middle frontal gyrus ROI in the dorsolateral prefrontal cortex was the only one to show a correlation with a physiological measure of successful regulation ($r = -.60$, $p < 0.02$). Specifically this correlation suggested that individuals with greater successful regulation,

indicated by a large difference between SCR data acquired during Attend CS+ and Regulate CS+ trials, also showed greater BOLD responses for Regulate, rather than Attend CS+ trials. Additionally, the differential response between Attend and Regulate CS+ trials in the middle frontal gyrus ROI correlated with the differential response between Attend and Regulate overall in the right striatum ROI ($r = -.53$, $p < 0.05$), suggesting a potential interaction between these regions during emotion regulation. Many emotion regulation studies have identified the dorsolateral prefrontal cortex as an important structure during emotion regulation, although there is considerable variability in the location within the PFC in general, typically engaged across different studies and emotion regulation strategies, ranging from active reinterpretation to more diversion based approaches (for review see [3]). For instance, in one study, self-focused regulation and situation-focused regulation, two different types of cognitive strategies, identified medial and lateral prefrontal ROIs, respectively [18].

Finally, another region that showed greater BOLD responses during Attend compared to Regulate trials was the subgenual cingulate cortex in the medial prefrontal cortex (BA 25), previously linked to fear extinction and regulation[11]. It is possible that an overlap exists between the mechanisms underlying natural extinction of the value of a conditioned stimulus, and those involved in the facilitation of extinction via other means (e.g., cognitive strategies).

Exploratory analysis: Interaction

A final whole brain exploratory analysis specifically identified voxels that showed an interaction between type of conditioned stimuli (CS+, CS–) and type of instruction (Attend, Regulate). As expected, activation of both left and right striatum, located in the more anterior and ventral portions of the caudate nucleus, was observed. This result supports the main findings that emotion regulation strategies efficiently modulate neural signals in the striatum during reward expectation.

One potential concern is that the observed effects are a result of increases or "up-regulation" in the Attend condition, rather than a decrease or "down-regulation" by the Regulate condition. The current physiological and subjective data suggests a decrease in emotional responses during the use of cognitive strategies. Additionally, the observed BOLD response in the striatum in response to the expectation of reward in the Attend condition is quite similar to a number of published studies examining this issue in absence of regulation instructions (e.g., [7,8,9], for review see [10]), with one particular paradigm showing increased SCRs [1] and BOLD responses [19] in a similar striatum ROI when participants are expecting a potential reward without instruction. Yet, without another control condition (perhaps aimed at increasing the current emotion), it is difficult to fully attribute the observed effects purely to "down-regulation". Future studies may investigate the potential contributions of "up-regulation", known to influence behavioral and neural responses in emotion regulation paradigms[18], during the expectation of reward, a phenomenon that could potentially lead to increased maladaptive decision-making.

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