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Neural biomarkers for assessing different types of imagery in pictorial health warning labels for cigarette packaging: A cross-sectional study.

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Abstract

Objective Countries around the world have increasingly adopted pictorial health warning labels (HWLs) for tobacco packages to warn consumers about smoking-related risks. Research on how pictorial HWLs work has primarily analyzed self-reported responses to HWLs; studies at the neural level comparing the brain's response to different types of HWLs may provide an important complement to prior studies, especially if self-reported responses are systematically biased. In this study we characterize the brain's response to three types of pictorial HWLs for which prior self-report studies indicated different levels of efficacy.

Methods Current smokers rated pictorial HWLs and then observed the same HLWs during functional magnetic resonance (fMRI) scanning. Fifty 18- to 50-year-old current adult smokers who were free from neurological disorders were recruited from the general population and participated in the study. Demographics, smoking-related behaviors, and self-reported ratings of pictorial HWL stimuli were obtained prior to scanning. Brain responses to HWLs were assessed using fMRI, focusing on a priori regions of interest.

Results Pictorial HWL stimuli elicited activation in a broad network of brain areas associated and visual processing and emotion. Participants who rated the stimuli as more emotionally arousing also showed greater neural responses at these sites.

Conclusions Self-reported ratings of pictorial HWLs are correlated with neural responses in brain areas associated with visual and emotional processing. Study results cross-validate self-reported ratings of pictorial HWLs and provide insights into how pictorial HWLs are processed.

Strengths and limitations of this study

- This is the first study to explore the relationship between self-reported ratings of pictorial HWLs and neural responses to pictorial HWLs in a large sample (N = 50) of current adult smokers.

- This paper demonstrates the amygdala is maximally activated by pictorial HWLs that depict human suffering, followed by images that depict graphic effects of smoking, followed by symbolic images of the negative consequences of smoking.
- This paper demonstrates that neural responses to pictorial HWLs attenuate with repeated exposure in most brain regions, but that this response is different in the amygdala.
- Further research is required in order to determine i) exactly why pictorial HWLs depicting human suffering elicited such robust responses in the amygdala and ii) whether differential adaptation to Symbolic stimuli is relevant to the creation of optimal HWLs.

INTRODUCTION

According to the World Health Organization, smoking remains the leading cause of preventable death in the Western world.(1, 2) Smoking increases the risk of many non-communicable diseases both in smokers and in those who breathe second hand smoke.(3)[3] To help prevent tobacco use and its consequences, the World Health Organization Framework Convention on Tobacco Control (WHO FCTC) has recommended including prominent, pictorial health warning labels (HWLs) on tobacco packaging to communicate the adverse effects of smoking to consumers and to discourage smoking.(2)Experimental and observational research indicate that HWLs with pictorial imagery are *more* effective than text-only HWLs in both promoting smoking cessation and preventing the initiation of smoking behavior.(4-7)[4–7] A key advantage of pictorial HWLs is likely due to their ability to elicit stronger emotional responses than text-only HWLs.(8)

The increasing adoption of pictorial HWLs around the world has created a critical need for research designed to i) evaluate the relative effectiveness of different types of HWL content and ii) explain why some HWL content appears more effective than other content. Such

research should guide the selection of HWL content, including the rotation of new HWL content over time. Some experimental research has found the self-reported effectiveness of pictorial HWLs is highest when it contains graphic images that depict the physical effects of smoking, followed by imagery of personal suffering (usually including a face), and finally by symbolic representations of smoking effects that use abstract imagery or symbols.(9-12)These findings are consistent with some observational studies indicating that graphic depictions of smoking consequences work best.(13, 14)

The *primary goal* of the current experiment was to explicitly map neural responses to HWLs that contain three different subtypes of imagery that are frequently used in tobacco control communications, including HWLs on cigarette packaging: graphic representation of physical consequences of smoking; personal suffering from smoking-related consequences; and symbolic representations of risk. Given the visual and emotional nature of pictorial HWLs, we formulated a set of *a priori regions of interest* (ROIs) that we expected to respond to participants' observations of HWLs, including the amygdala, insula and visual cortex. Converging evidence from numerous neuroscientific investigations confirms a prominent role for the *amygdala* in emotional processing in a number of sensory modalities.(15-19) The amygdala plays a particularly important role in the processing of visual stimuli related to threat and fear.(20-22) We expected that amygdala responses would be driven by our stimuli to the extent that they elicited arousal, fear and perceived threat (e.g., graphic HWL vs. symbolic HWL). We also expected pictorial HWLs to elicit robust activity in the *insula*. This area has been linked to the experience of disgust, and strongly responds to pictures of mutilation and contamination.(23-26) Finally, based on a prior investigations of the neural response to emotional pictures, we expected the *visual association cortex* to be robustly activated by the presentation of pictorial

HWLs.(27-29) We expected all three subtypes of HWLs to elicit a significant response (relative to rest) in this subset of *a priori* regions of interest.

Our *secondary goal* was to examine the relationship between self-report data indicating that HWLs that use graphic imagery are more effective than HWLs depicting human suffering, which were in turn more effective than symbolic HWLs. We hypothesized that the neural response in our *a priori* regions of interest would differentiate between our three types of HWL (Graphic > Suffering > Symbolic), and that participants who rated pictorial HWL stimuli as more emotionally arousing exhibit heightened activity in these areas. In order to examine these questions, 50 current adult smokers self-reported emotional arousal of HWLs of each pictorial subtype and subsequently observed the same stimuli while their brain activity was measured using fMRI.

METHOD

Participants

Fifty adult smokers between the ages of 18 and 50 (24 females, Mean Age = 27.57) took part in this study. Participants were recruited from the general public, via fliers posted in public locations around the University of South Carolina (USC) and local newspapers. All participants were neurologically healthy smokers with normal to corrected vision. Following initial phone and online screening to confirm qualification for participation, all subjects reported to the McCausland Center and provided informed consent prior to MRI scanning. Following completion of the study protocol, participants were paid $100 for transportation costs related to participation in the study. The experiment was performed according to the guidelines of the Declaration of Helsinki and approved by the IRB at USC.

Pictorial HWL Stimuli

A total of 57 pictorial HWLs were used, with images drawn primarily from, based on, or considered for actual HWLs implemented in different countries (**Supplementary Material)**.(6, 30, 31) Nineteen pictorial HWLs were developed for each of three pictorial styles: 1) Graphic health effect - vivid depiction of physical effects of smoking on the body; 2) Human suffering - depiction of personal experience which shows the face and could include the physical, social or emotional impact of smoking-related harm and; 3) Symbolic – representation of message using abstract imagery or symbol. HWL textual content involved short, factual statements based on HWLs that have been implemented and used in prior research.(9)Textual accompaniments addressed 13 different health topics were addressed (i.e., addiction, death, emphysema, gangrene, heart disease, lung cancer, mouth cancer, pregnancy, breast cancer, second hand smoke, strokes, throat cancer, and blindness), with some topics repeated twice within categories (emphysema, death, heart disease, lung cancer, mouth cancer, stroke) Topics and text were counterbalanced across the three pictorial styles. Importantly, the mean luminance values for pictorial HWL s did not differ between subtypes (all p's > 0.18), nor did the overall color (as measured by Red, Green or Blue color values) (all p's > 0.11).

Measures

Demographics

All participants were asked a series of standard questions regarding their age, gender, income, ethnicity, and current and past use of cigarettes (**Table 1)**.

Self-reported responses to HWLs

Prior to attending the laboratory session, each participant completed a short survey and rated all 57 HWLs, which were presented online and in random order. Negative emotional

arousal was assessed by asking participants to rate the HWL on how much it made them afraid ("How much does this warning make you feel afraid?"). As in prior research, (9, 12) participants were also queried concerning ad effectiveness ("How effective is this warning?). For both questions, participants responded with a rating of 1 to 9, with verbal anchors at either end of the rating scale (i.e., 1 = not at all, 9 = extremely).

Neural response to HWLs

During 50 minutes of MRI scanning, each participant completed a single, high resolution structural scan, as well as four functional MRI task runs. Each functional run was 10 minutes and 24 seconds in duration. During each functional run, each of the 57 images (19 Graphic images, 19 Suffering images and 19 Symbolic images) was presented a total of 10 times each. These images were presented using a block design format. Each block of stimuli was 15 seconds in duration and consisted of the serial presentation of 5 images from the relevant condition (or fixation cross for Rest), separated by 1 second of fixation. A total of 40 blocks (10 Graphic, 10 Suffering, 10 Symbolic and 10 Rest) were presented during each of four functional runs. (Figure 1) The order of presentation of the blocks within a given functional run was chosen from one of eight pseudo-randomly generated trial orders. These orders were constrained such that i) each condition was equally likely to follow any other condition within a certain functional run; and ii) blocks of the same trial type never occurred more than three times in a row. Each of the four functional runs was identical in duration and content with the exception of the random assignment of images from each condition to its corresponding block. Importantly, the total time (and thus total number of brain volumes recorded) spent showing blocks of each picture type was identical to the total time spent showing Rest blocks.

In order to ensure that participants paid attention to the visual stimuli, we employed a 1-back picture recognition task. Participants were instructed to press a button when the same picture appeared twice in a row. This occurred 5 or 6 times (randomly chosen to prevent participants from assuming they were done detecting repeats within a given run) during each functional run. Placement of repeats was randomized prior to each run using Presentation's built in randomization features.

Procedures

Smoking Status Screening

In order to confirm that participants were indeed current smokers they underwent screening prior to scanning. Carbon monoxide (CO) levels were measured in all participants immediately prior to scanning using a piCO+ Smokerlyzer (Bedfont Scientific, Harrietsham, England). All participants also provided saliva samples immediately prior to scanning. Saliva sample collection involved placing a cellulose pad affixed to a polypropylene stem (collector) under the participant's tongue until a defined volume of saliva saturated the cellulose pad. These samples were sent to Labcorp/MedTox laboratories where cotinine (nicotine metabolite indicating tobacco smoke exposure) was assessed using liquid chromatography with Tandem Mass Spectrometry (LC-MS/MS). Participants also reported the time since last cigarette, the number of days they smoked in the last 30 days, and the average number of cigarettes they smoked per day during that time (**Table 1**).

Image acquisition

All MRI data were collected on a 3T Siemens Trio system with a 12-element head coil. The fMRI (T_2* echo planar imaging) imaging sequence included the following parameters: 320 full brain volumes collected in each of the four 10-minute, 30-second sessions; 75° flip angle;

time repetition (TR) = 1.95 s; time echo (TE) = 30 ms; in-plane resolution 3.30 × 3.30 mm; slice thickness = 3.0 mm (no gap); 36 axial slices collected in planes aligned parallel to the anterior commissure–posterior commissure line. To improve coregistration of images, all participants were scanned with a high-resolution T_1 MRI, which yielded a 1-mm isotropic image. This sequence had the following parameters: field of view (FOV) = 256 × 256 mm, 192 saggital slices, 9° flip angle, TR = 2250 ms, TE = 4.15 ms.

Data Preprocessing and Modelling

All fMRI data were preprocessed and analyzed using SPM8 (Wellcome Department of Cognitive Neurology, London). Standard preprocessing procedures included image realignment (4^{th} Degree B-Spline Interpolation), coregistration (Mean EPI aligned with T1 then parameters applied to all EPIs), normalization and spatial smoothing (Gaussian Kernel FWHM 8mm). The onsets and durations of each of the conditions of interest were modeled according to the block design described in the protocol. At the first-level, functional data was modeled as a boxcar canonically convolved hemodynamic response function (duration 15 sec). For all group analyses reported below, we first generated a series contrast images for each individual participant (first level models) and then entered these into random effects models and/or regression models (using SPM's built in general linear model) in order to allow for meaningful population-level inference. First eigen-variates were extracted from second-level models (for each ROI/condition/session) using the VOI toolbox in SPM 8.(32) The resulting parameter estimates were used as the primary dependent variables in the statistical models reported below (i.e. ANOVA and regression analyses).

RESULTS

Behavioral Performance

Population Variables

Our Participants in the current study were equally spilt with respect to gender (52% Male, 48% Female) and predominantly white (74%, 24% African American, 2% other). The majority of participants (55%) had at least some post-high school education, and were low-income. At the time of scanning, the group's CO levels were 18.74 ppm and cotinine was measured at 207.48 ng/mm confirming that all participants were active smokers. Furthermore, the average participant smoked 18.74 cigarettes per day, and reported having smoked on 28.32 out of the previous 30 days.

Self-reported Ratings of HWLs

Differences in self-reported emotional arousal across the three stimulus types (Graphic, Suffering, Symbolic) was assessed using one-way within subjects ANOVA, $F(1.44,70.53) = 121.01$, $p < 0.001$. All post-hoc pair-wise comparisons were significant differences between ratings of Graphic ($M = 5.14$, $SD = 1.98$) and Suffering ($M = 4.02$, $SD = 1.82$) stimuli, as well as between ratings of Suffering and Symbolic ($M = 2.39$, $SD = 1.43$) stimuli. A one-way within subjects ANOVA using perceived effectiveness as a dependent variable and stimulus-type (Graphic, Suffering, Symbolic) as the dependent variable was also significant, $F(1.54,75.27) = 133.27$, $p < 0.001$. Responses to the emotional arousal and perceived effectiveness questions were highly correlated for the Graphic ($r(49) = .87$), Suffering ($r(49) = .90$) and Symbolic ($r(49) = .90$) stimuli. Because ratings of emotionality were the most relevant for interpretation of our results, we focus on those scores in our analysis section. We would like to note that we did perform the same analyses using perceived effectiveness and obtained a similar pattern of results. (**Figure 2**)

fMRI One-back Task:

One-back task performance data was collected from a total of 176 out of 200 possible fMRI scanning runs (50 participants, with 4 runs per person). A one-way ANOVA using *error rate* as the dependent variable with session as the factor was not significant, $F(3,162) = 1.003$, $p = 0.393$. Moreover, post-hoc comparison of all possible session pairings failed to reveal any significant differences in 1-back performance between n any two sessions (all p's > 0.33).

fMRI Response

Main Effects of Pictorial HWLs on Neural Response

In order to isolate cortical networks activated by the presentation of each type of pictorial HWL, we computed a series of contrasts designed to test for the main effects of each of the three stimulus types (Graphic, Suffering, and Symbolic). Specifically, we computed the following contrasts: Graphic-Rest, Suffering-Rest and Symbolic-Rest (thresholded at $p < 0.05$ and corrected for family-wise error (FWE)). Observation of pictorial HWL stimuli elicited a significant neural response in a broad network of brain areas including our a priori regions of interest (the amygdala, insula, and visual association cortex) as well as a number of other brain areas including the frontal gyrus (inferior, middle, medial, and superior aspects), temporal gyrus (middle and superior), parietal lobe (inferior), supplementary motor area, parahippocampal gyrus, and thalamus. The results of this analysis are listed in **Tables 2-5** and displayed graphically in **Figure 3**.

We performed additional analyses in order to identify brain areas whose response properties showed the same pattern as participants' self-reported evaluations of the experimental stimuli in each group (Graphic > Suffering > Symbolic). Accordingly, we performed ROI analyses on our apriori regions of interest including the amygdala, insula and secondary visual

cortex. Regions of interest within the visual association cortex, amygdala and insula were created based on peak activations observed in the contrast comparing the brain's response to all conditions to rest ([Graphic + Suffering + Symbolic] – Rest). All ROIs were centered at the site of peak activation within a given ROI and were spherical in nature (r = 4 mm). A series of one-way within-subjects ANOVAs were used to evaluate neural responses patterns (for Graphic, Suffering and Symbolic stimuli) within our ROIs. These ANOVAs were significant in the left amygdala, $F(2,98) = 14.59$, $p < 0.001$, right amygdala, $F(2,98) = 21.60$, $p < 0.001$, left insula, $F(2,98) = 4.42$, $p < 0.05$, and visual association cortex, $F(2,98) = 22.69$, $p < 0.001$. As with the behavioral data, we conducted post-hoc pairwise comparisons (all significant results were $p < 0.05$, Bonferroni corrected). In the left amygdala we observed a significant difference between responses in the Graphic and Symbolic conditions, as well as in the Suffering and Symbolic conditions. In the right amygdala all pair-wise comparisons were significant. In the left amygdala and the visual association cortex, responses to Graphic and Symbolic stimuli were significantly different, as were responses to Graphic and Suffering stimuli. The results of these analyses are shown graphically in **Figure 4, A.** We also conducted whole-brain analyses for the following direct comparisons between conditions: Graphic > Symbolic, Symbolic > Graphic, Suffering > Symbolic, Symbolic < Suffering, Suffering > Graphic and Graphic > Suffering (See **Supplementary Materials**).

In addition to examining the main effects of stimulus type, we also conducted a series of 3 (Stimulus) x 4 (Session) repeated measures ANOVAs (one for each ROI) in order to explore possible BOLD signal adaptation to our three stimuli types across the four fMRI sessions. The main effect of session was significant for the left insula, $F(3,138) = 11.40$, $p < 0.001$, right insula $F(3,138) = 3.19$, $p < 0.05$, and visual association cortex, $F(3,138) = 15.43$, $p < 0.001$, and nearly

significant in the left amygdala, $F(3,138) = 2.66$, $p = 0.74$. There was a significant interaction between Stimulus and Session in both the left amygdala, $F(6,276) = 2.28$, $p < 0.05$, and right amygdala, $F(6,276) = 2.15$, $p < 0.05$. These results are shown split by session (in order to visualize adaptation) in **Figure 4, B**.

We also ran a series of targeted correlations to determine whether there was a relationship between individual ratings of pictorial HWLs of specific subtypes and the BOLD signal elicited by their presentation. For the graphic stimuli, we conducted an SPM multiple regression analysis using individual contrast images for the Graphic-Rest condition as the dependent variable and mean self-reported arousal ratings for the Graphic HWLs as the independent variable (thresholded at $p < 0.001$, 5 voxel extent). Similar regression analyses were conducted to examine the correlation between HWL ratings and BOLD signal in the Suffering and Symbolic conditions. In all three analyses, activation in the right visual association cortex (XYZ_{mni} = -18, -92, 20, XYZ_{mni} = -20, -88, 12, and XYZ_{mni} = -14, -92,12 respectively) was positively correlated with mean ratings of the pictorial HWLs (all r(49)'s > .48) (**Figure 5**). For graphic and suffering HWLs additional positive correlations were found at sites in the right precentral gyrus (XYZ_{mni} = 44,4,40), r(49) = .45 and r(49) = .42 respectively. For symbolic HWLs there was an additional positive correlation between HWL ratings and activation in the left inferior frontal gyrus (XYZ_{mni} = -52,16,30), r(49) = .37).

DISCUSSION

The present study explicitly measured neural responses to observation of pictorial HWLs in a population of confirmed cigarette smokers. Results indicated that pictorial HWLs of all types elicited activation in areas associated with visual processing, as well as the processing of fear and disgust. Activation at sites in the inferior frontal gyrus/precentral gyrus, visual cortex,

and to a lesser extent the insula, showed a pattern for strength of response by pictorial stimulus type (i.e., Graphic > Suffering > Symbolic) that was the same as was found for participants' self-reported ratings of the fear elicited by the stimuli. However, amygdala responses appeared more complex, and it responded maximally to pictorial HWLs depicting human suffering, perhaps due to its involvement in empathetic responses (see below). Previous experimental research has found that HWL imagery that combines human suffering with graphic imagery is rated as more effective than either imagery type alone.(9) In many cases the suffering imagery used in our study included graphic elements, and that combination may most effectively promote amygdala response. Finally, for all pictorial HWLs, participants that perceived the pictorial HWLs as particularly effective showed heightened activation in the visual association cortex.

Main Effects of HWL Type

The primary goal of the current experiment was to measure the neural response to presentation of pictorial HWLs. Based on prior literature mapping the brain's response to vivid graphic images, we expected the more graphic HWLs to elicit activation in the amygdala, and insula. Our results are consistent with this literature in that all subtypes of pictorial HWLs used in the current study elicited activation at sites in the amygdala, the insula and the visual association cortex.

Region of Interest Analysis

A secondary goal of this experiment was to examine the relationship between self-reported ratings of pictorial HWLs with brain data. We expected that responses in regions associated with visual and emotional processing would mirror self-reported ratings of the stimuli (i.e. Graphic > Suffering > Symbolic). Results from our ROI analysis were partially consistent

with this prediction. Activity in the right visual association cortex did scale in the same manner as self-reported ratings. The more vivid/graphic nature of certain subtypes of pictorial HWLs may be responsible for the differences we observed in the visual cortex. Images in the Graphic condition contained more gory/bloody elements than those in any of the other two conditions, and the images in the Suffering condition contained a moderate amount of these elements. It is well established that the amygdala, a key neural pathway for responses to graphic imagery, projects to both primary and secondary visual cortices.(33) It is unlikely that this activation was caused by differences in low-level features of the images because luminance and color values were not significantly different for the three HWL subtypes. Additionally, in at least one previous experiment examining the impact of arousing visual stimuli on visual cortex activity, differences in eye movements did not account for the observed patterns of activation.(28) Therefore it is unlikely that the effects we report were due to differential eye movements.

While responses in the visual association area and insula were at minimal consistent with self-reported ratings, activation patterns observed in amygdala were not. Surprisingly, the amygdala was most robustly activated by Suffering HWLs, followed by Graphic HWLs, and finally Symbolic HWLs. As noted in the introduction, the amygdala has been shown to be responsive to arousing stimuli, and fear-evoking stimuli appear to be particularly potent at activating this brain structure. One possibility, then, is that the HWLs that depict personal suffering from smoking-related outcomes are best at eliciting fear. However, this is inconsistent with the self-reported data, which indicated that Graphic HWLs elicited maximal fear responses. A more parsimonious explanation for this finding is that the relative hyper-activation observed for HWLs with Suffering imagery was due to the presence of human faces in the stimuli (all 19 Suffering HWLs contained human faces). Lesion, single-cell and whole brain neuroimaging

experiments are consistent with the idea that the amygdala is a key component of the face-perception network.(18, 34-39) The amygdala may even process fearful facial stimuli in the absence of conscious processing.(40, 41) Faces may be particularly important under conditions of repeated exposure, as with HWLs, as we may be drawn to faces even after repeated exposure, whereas we may be less drawn to graphic bodily harm. Some of the suffering images (4 of 19) portrayed visible body damage, and so Suffering imagery was not entirely distinct from graphic imagery used. To better isolate any differential effects of these two image types and the interaction between them, future studies should use imagery that more clearly falls into one category, the other, or both. Another possible explanation for the increased relative amygdala activation observed in the Suffering condition relates to stimulus salience. Studies have demonstrated a strong link between amygdala activation and stimulus salience.(42, 43) In the context of the current experiment, it may be that images depicting smoking-related suffering were particularly salient to current smokers. While this could have implications for the optimization of HWLs, further experimentation is necessary to evaluate this hypothesis. Future research should aim to separate out the effects of emotionality, salience and human faces by integrating additional conditions (such as neutral images with and without faces). Based on research demonstrating the that BOLD signal in the amygdala is a predictor of subsequent quitting behavior (44) (as is BOLD signal in the medial prefrontal cortex (45, 46)), it might be useful to conduct future prospective studies that examine the extent to which amygdalar BOLD response to the three types of HWLs discussed in the current paper predict changes in smoking behavior.

To the extent that HWL effectiveness depends on enduring emotional responses, neural adaptation to repeated exposure is an important issue to consider. Our exploratory, post-hoc

analysis of region-specific adaptation revealed that, in the majority of our regions of interest, BOLD response decreased as a function of repeated exposure to all HWLs. Interestingly, we observed a significant deviation from this pattern in the left and right amygdala. While activation associated with observation of Graphic and Suffering images consistently decreased across the four sessions, activation patterns associated with observation of Symbolic images were less consistent (**Figure 4, B**). It is tempting to speculate that participants did not adapt (neutrally speaking) to repeated presentation of Symbolic stimuli in the same way they adapted to images in the Suffering and Symbolic categories. The abstract nature of these stimuli may have necessitated additional exposure in order to fully process their meaning, and this may account for the observed findings. These data should be interpreted cautiously as repeated exposure to HWLs during three, 10-minute scanning sessions may not accurately mimic repeated exposure to HWLs as it exists in real-life (temporally spread out, situation specific, craving-state specific, etc.). Further scrutiny of neural adaptation across repeated sessions or repeated days could isolate differences in neural adaptation. If these neural responses can be linked to changes in smoking behavior, public health could be positively impacted.

Relationship Between Neural Measures and Self-Report Data

An important goal of the present study was to cross-validate self-reported ratings of pictorial HWLs and brain activity recorded during the observation of the same stimuli. This paper is the first to report such results for smoking HWL stimuli. In general, our correlational data indicate that participants who rated pictorial HWL stimuli (within each category – as opposed to between categories) as more emotionally arousing showed higher activation of the visual association cortex when viewing the stimuli. This finding is consistent with previous reports demonstrating that activity in the visual cortex is particularly robust during the

presentation of emotionally arousing visual stimuli, perhaps due to reentrant enhancement of V2 activity being driven by motivational processes that heighten input from the amygdala. (27, 29, 47)[27–29]

We also observed an unexpected correlation between self-report ratings and activity at the junction of the right precentral gyrus and inferior frontal gyrus (pars opercularis). Given the location of the activation in the RH (as opposed to the LH which is traditionally associated with such language functions), it is unlikely that heightened responses reflect increased reliance on language. This site is considered to be part of the human mirror neuron system (MNS) and thought to interact with the amygdala and insula when we establish a link between the actions/emotions/intentions of others and our own actions.(48)[49] These stimuli may have been particularly effective at eliciting the types of interpersonal comparisons and or emotions (i.e. empathy) that individuals typically make when seeing the negative effects of their own behaviors in others.(46, 49-51)[49–52] Another possible explanation for the significant correlation we observed between right IFG activity and self-reported ratings is that more emotionally arousing stimuli required greater emotion regulation on the part of the observer. This is consistent with studies reporting recruitment of the right IFG during tasks that require the inhibition of emotions. (52-54)

Finally, we observed a significant relationship between activity in the left inferior frontal gyrus (BA 44) and self-report ratings of the symbolic stimuli. This area has traditionally been associated with language processing and is active during both overt (i.e. spoken) and covert (i.e. silent) speech.(55-59)[53–57] It is not surprising that symbolic stimuli would utilize language processes. Stimuli of this subtype were the most abstract and likely evoked covert speech during the interpretation process. These data suggest that the Symbolic HWL stimuli that maximally

engage language processes are likely to be rated as more arousing than those that do not. If symbolic stimuli are too abstract/confusing to easily verbalize (covertly), then they may be interpreted as more fear eliciting. The involvement of language areas during HWL processing could be the topic of future experiments that assess verbalization during presentation of HWLs of all types.

While we did not find significant correlations between amygdala activity and self-reported ratings of arousal (as might be expected), the correlation between BOLD signal in the right amygdala and self-reported responses in both Graphic ($r(49) = .21$, $p = 0.07$ one-tailed) and Suffering ($r(49) = .20$, $p = 0.08$ one-tailed) conditions was nearly significant, and in the predicted direction. It is useful to consider why this correlation might have failed to reach statistical significance. One possibility for this negative finding is that the amygdala's response to the emotional stimuli was blunted by the inclusion of text in the HWLs used in the present study. This interpretation is consistent with a comprehensive meta-regression analysis of imaging studies reporting amygdala activation which found that presence of language in the stimulus was associated with reduced amygdala activation (as well as greater left lateralization relative to baseline).(60) This finding is particularly interesting in light of trends towards the adoption image based HWLs. While the inclusion of text in graphic warning labels has traditionally been justified in terms of added information content (text adds information otherwise not present), it may also be important to examine possible emotional 'blunting' effects that inclusion of text may have. Future brain imaging could further

Summary

The present study examined self-reported and neural responses to pictorial HWL stimuli of three different types in a population of current adult smokers. Pictorial HWLs elicited robust

responses in a broad network of brain sites including those associated with image interpretation (visual association cortex) and emotion (amygdala and insula). Moreover, activation in visual, premotor, inferior frontal and to a lesser extent the insular areas, varied in the same manner as self-reported ratings of the stimuli. We found a robust relationship between neural responses which is important given that self-reported data are subject to numerous forms of bias. Nevertheless, both methods should be assessed for their predictive validity (i.e., prediction of smoking cessation), because pictorial HWL exposures under experimental conditions are likely to be different from when smokers are repeatedly exposed in more mundane contexts to the same pictorial HWLs over time. Brain imaging provides insights into the neural pathways through which pictorial HWLs influence behavior. For example, in the current study, the amygdala was most active in response to HWLs depicting human suffering, which was contrary to expectations. Future research should more directly examine the relationship between the strength of brain activity elicited by specific subtypes of pictorial HWLs (at multiple sites) after repeated exposures to HWLs, and the likelihood of behavior change, whether measured as actual smoking cessation or other behavioral proxies of cessation.

Table 1.

Demographic and Smoking Behavior Information		
Demographic Variables		n = 50, mean (SD) or %
sex	% female	48%
age	Mean	27.56
	Range	22
race	% White	74%
	% African American	24%
	% Other	2%
Education	High school or less	26%
	some college/tech school	55%
	college or more	18%
Income	low	63%
	middle	30%
	high	7%
Smoking/Consumer Behavior		
CO Level (ppm)		18.74 (10.57)
Cotinine Level (ng/mm)		207.48 (173.27)
Days Smoked (last 30 days)		28.32 (4.63)
Cigarettes (per day)		14.90 (10.09)
How worried smoking affects health?	not at all	0%
	a little worried	48%
	very worried	52%
Pay attention to HWLs	not at all	54%
	a little worried	40%
	somewhat	4%
	a lot	2%

Tbl. 1. Demographic and behavior information.

Table 2.

region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
ALL - Rest:					
Lingual Gyrus	R	24	-90	-6	21.62
Fusiform Gyrus	R	42	-80	-10	19.48
Calcarine	R	12	-94	0	19.02
Hippocampus	R	20	-30	0	15.8
Hippocampus	L	-22	-30	-2	13.73
IFG Pars Triangularis	L	-52	24	30	9.87
Precentral Gyrus	L	-46	-4	52	9.71
Precentral Gyrus	L	-42	8	32	9.26
SMA	L	-6	8	56	8.99
SMA	R	6	10	52	8.53
IFG Pars Triangularis	R	48	24	26	8.67
IFG Pars Opercularis	R	54	22	32	8.66
Middle Frontal Gyrus	R	50	36	24	8.64
Insula	L	-30	28	2	8.39
IFG Pars Orbitalis	L	-34	30	-8	8.17
IFG Pars Orbitalis	L	-40	26	-12	7.81
Amygdala	R	20	-6	-14	7.33
Amygdala	L	-22	-4	-14	6.47
IFG Pars Orbitalis	R	28	30	-10	6.12
Insula	R	32	30	2	5.57
Fusiform Gyrus	L	-32	-32	-16	6.02
Parahippocampal Gyrus	L	-14	-28	-16	5.13

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute
T-value: local maxima thresholded at $p < 0.05$ FEW corrected, extent threshold $k = 10$

Tbl. 2. Table of brain activations elicited by observation of pictorial health warning labels collapsed across subtype ([Graphic, Suffering, Symbolic] vs [Rest]).

Table 3.

region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Graphic - Rest:					
Lingual Gyrus	R	24	-90	-6	19.86
Declive	L	-38	-70	-10	19.05
Fusiform Gyrus	R	42	-80	-10	18.41
Hippocampus	L	-22	-30	-2	11.35
Hippocampus	R	22	-30	0	13.19
Precentral Gyrus	L	-46	-4	48	9.42
Precentral Gyrus	L	-50	6	38	8.68
Precentral Gyrus	L	-42	6	32	8.47
SMA	L	-6	6	58	8.54
SMA	R	6	10	52	7.87
Precentral Gyrus	R	46	8	34	8.36
Middle Frontal Gyrus	R	50	36	24	8.31
IFG Pars Opercularis	R	54	22	30	7.91
Insula	L	-30	30	-4	7.46
Parahippocampal Gyrus	R	36	-6	-26	6.54
Amygdala	L	-22	-2	-16	6.38
Amygdala	R	22	-4	-14	6.1
Parahippocampal Gyrus	L	-30	-34	-16	5.94
IFG Pars Orbitalis	R	28	30	-10	5.69
Middle Temporal Gyrus	L	-54	-46	8	5.42

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute
T-value: local maxima thresholded at $p < 0.05$ FEW corrected, extent threshold $k = 10$

Tbl. 3. Table of brain activations elicited by observation of pictorial health warning labels of the subcategory 'Graphic' relative to Rest (i.e. fixation).

Table 4.

region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Suffering - Rest:					
Fusiform Gyrus	R	42	-80	-10	19.19
Lingual Gyrus	R	24	-90	-6	19.1
Occipital Lobe (Middle)	L	-26	-96	8	18.46
Hippocampus	R	24	-28	-2	15.59
Hippocampus	L	-22	-28	-4	14.41
Amygdala	R	20	-6	-14	9.36
IFG Pars Triangularis	R	52	30	26	9.05
IFG Pars Opercularis	R	46	14	32	8.54
IFG Pars Opercularis	R	52	20	34	7.88
Insula	L	-30	28	0	8.65
Inferior Frontal Gyrus	L	-36	20	-18	5.25
Precentral Gyrus	L	-46	-4	48	8.48
Precentral Gyrus	L	-40	8	32	8.42
IFG Pars Triangularis	L	-44	18	26	7.72
SMA	R	6	10	52	8.14
Amygdala	L	-20	-6	-14	7.71
Superior Temporal Gyrus	L	-52	-52	10	7.4
Insula	R	30	32	-8	6.31
Inferior Parietal Lobule	L	-48	-26	52	5.56
Superior Temporal Gyrus	R	48	-40	10	5.40

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute
T-value: local maxima thresholded at $p < 0.05$ FEW corrected, extent threshold $k = 10$

Tbl.4. Table of brain activations elicited by observation of pictorial health warning labels of the subcategory 'Suffering' relative to Rest (i.e. fixation).

Table 5.

region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Symbolic - Rest:					
Lingual Gyrus	R	24	-90	-6	19.56
Cuneus	L	-18	-100	6	18.61
Lingual Gyrus	R	12	-94	0	17.98
Hippocampus	R	22	-28	-2	14.14
Hippocampus	L	-22	-30	-2	11.36
IFG Pars Triangularis	L	-50	22	30	8.92
IFG Pars Opercularis	L	-42	10	30	8.57
Precentral Gyrus	L	-46	-4	48	8.5
SMA	L	-4	8	56	8.77
SMA	R	6	12	52	8.72
IFG Pars Opercularis	R	54	22	32	7.68
Middle Frontal Gyrus	R	50	36	24	7.59
Precentral Gyrus	R	46	12	32	6.76
Insula	L	-30	28	0	7.28
IFG Pars Orbitalis	L	-36	28	-10	7.2
Inferior Parietal Lobule	L	-46	-38	54	6.19
Inferior Parietal Lobule	L	-48	-28	52	5.32
Insula	R	32	30	2	5.2

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute
T-value: local maxima thresholded at $p < 0.05$ FEW corrected, extent threshold $k = 10$

Tbl. 5. Table of brain activations elicited by observation of pictorial health warning labels of the subcategory 'Symbolic' relative to Rest (i.e. fixation).

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Competing Interests Statement

The authors and coauthors have no competing interests to declare.

Contributorship Statement

Roger D. Newman-Norlund helped design the experiment, collected MRI data, analyze behavioral and MRI data, conducted statistical analyses and drafted the paper. James F. Thrasher oversaw the experiment, helped design the experiment, and helped draft the paper. Johann Fridriksson helped design the experiment, recruited participants, collected behavioral and MRI data and revised the draft paper. William Brixius helped collect MRI data and revised the draft paper. Brett E. Froeliger, David Hammond and Michael K. Cummings helped design the experiment and draft the paper.

Data Sharing Statement

All data collected in this experiment is located on the hard drive of the corresponding author, R.D.N. Data analysis is ongoing and the data may still be used for additional papers. After all planned papers have been submitted, the data may be made available to others, upon written request, from R.D.N.

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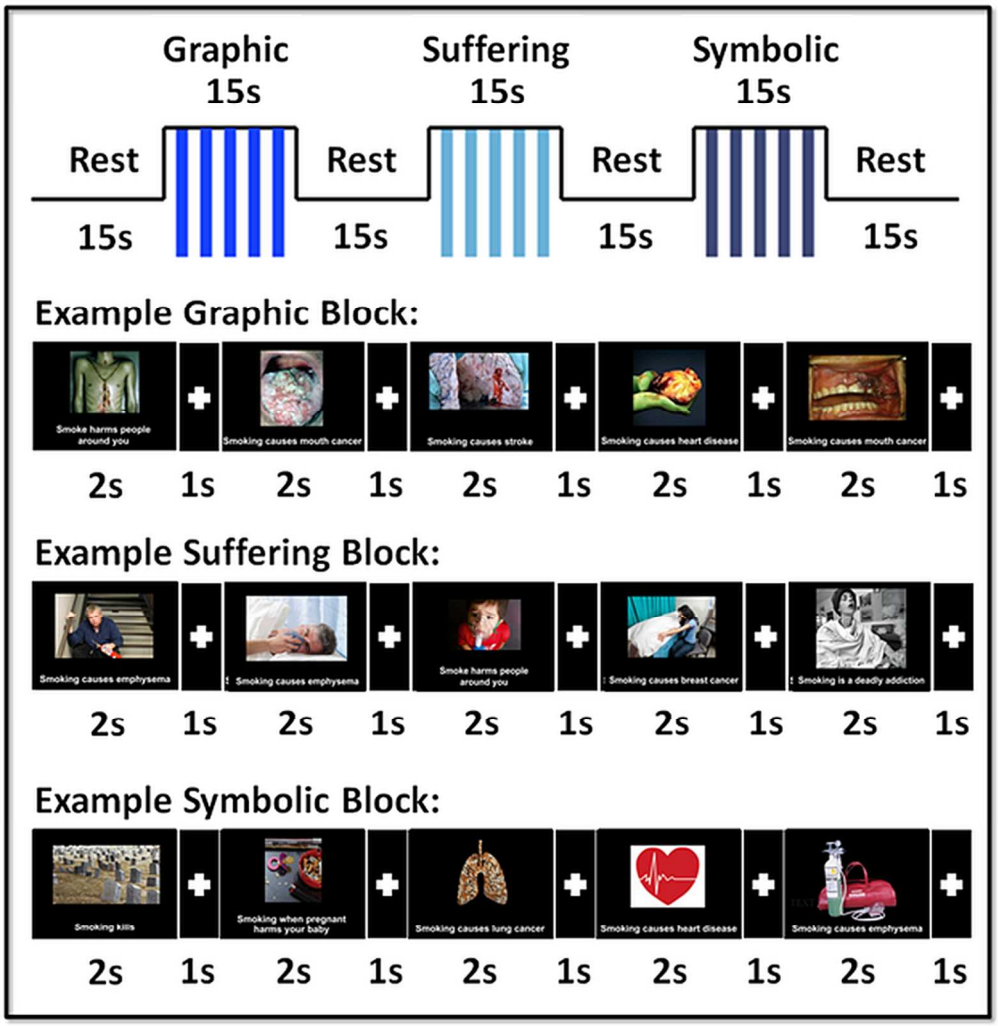

Figure 1. Graphical representation of the construction of each functional run. All stimuli types (Graphical, Suffering, and Symbolic) were presented in block format. Each block consisted of the presentation of five pseudo-randomly selected stimuli of the appropriate type presented for 2 seconds each, and separated by 1 second of fixation. Block order was pseudo-randomized for

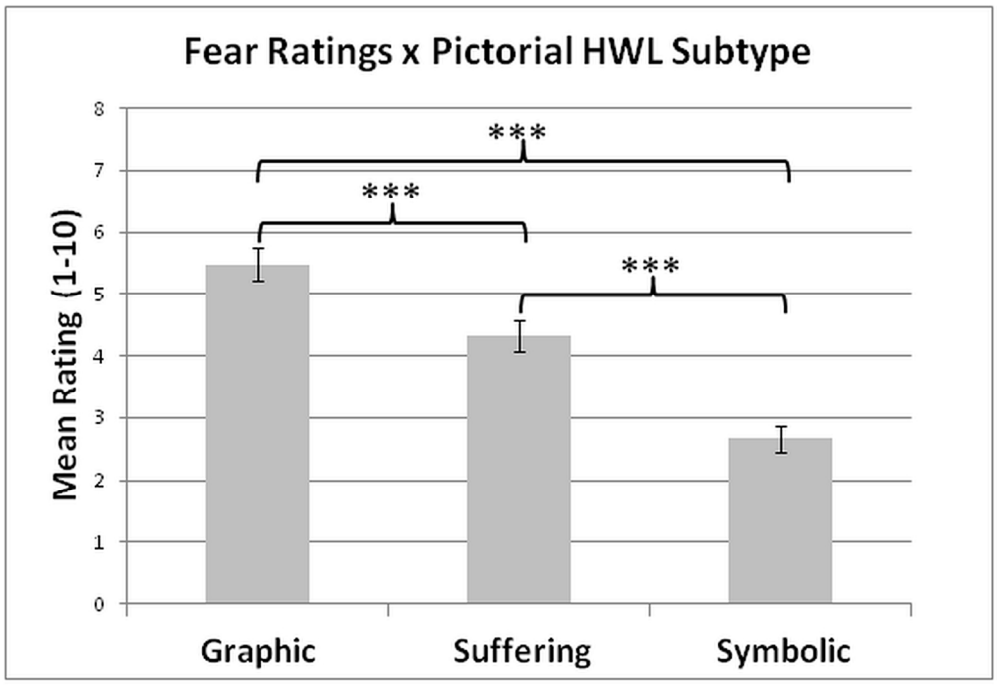

Figure 2. Behavioral effectiveness ratings of HWLs. All participants rated all HWL's prior to fMRI scanning by responding to the question: "How much does this warning make you feel afraid?" Error bars represent standard error of the mean (SEM); *** = significant $p < 0.001$ (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM).

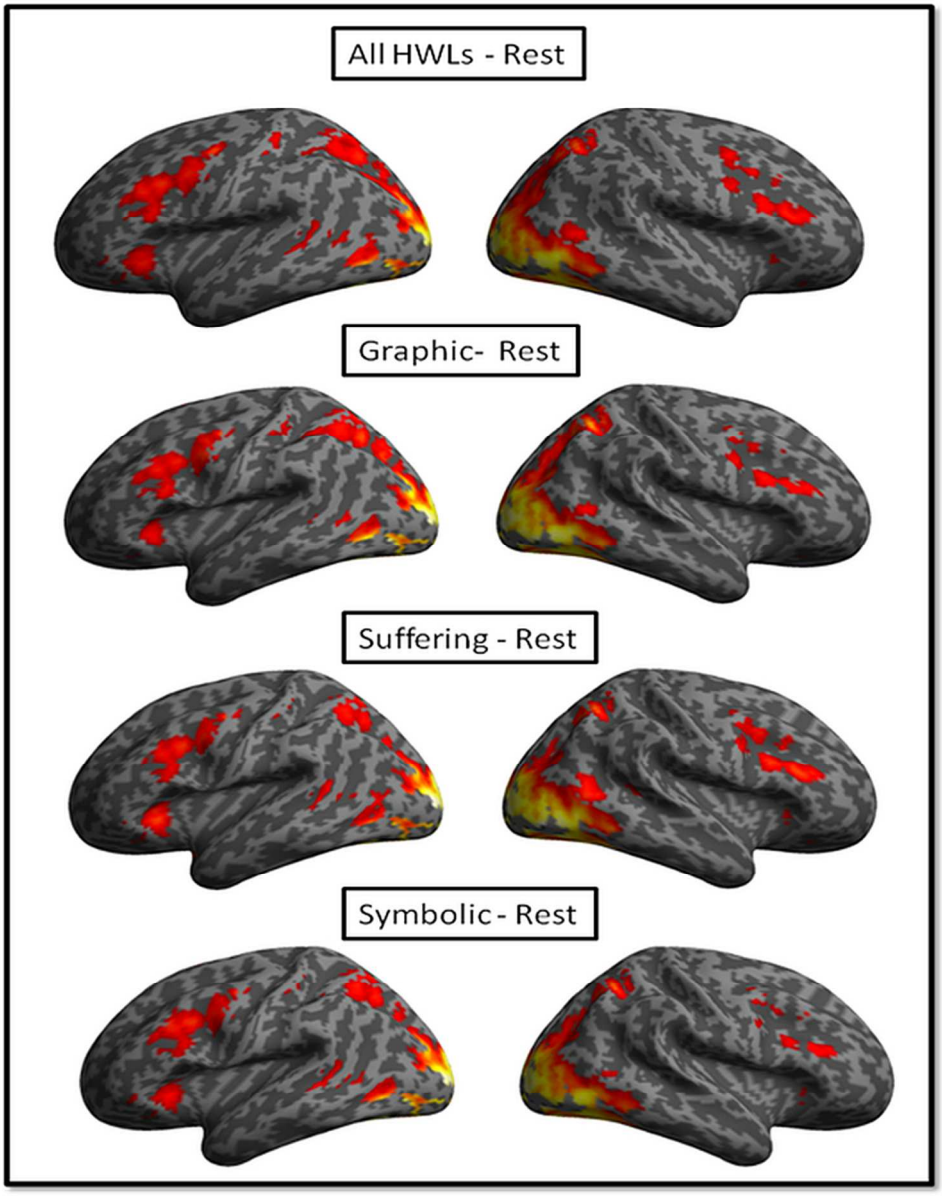

Figure 3. Main effects of HWLs on BOLD signal (Graphic, Suffering, Symbolic) on BOLD signal. All results are thresholded at p < 0.05 and corrected for family-wise error (FWE). Results are overlaid on a standard inflated brain (cortex_20484.surf.gii) for illustration purposes.

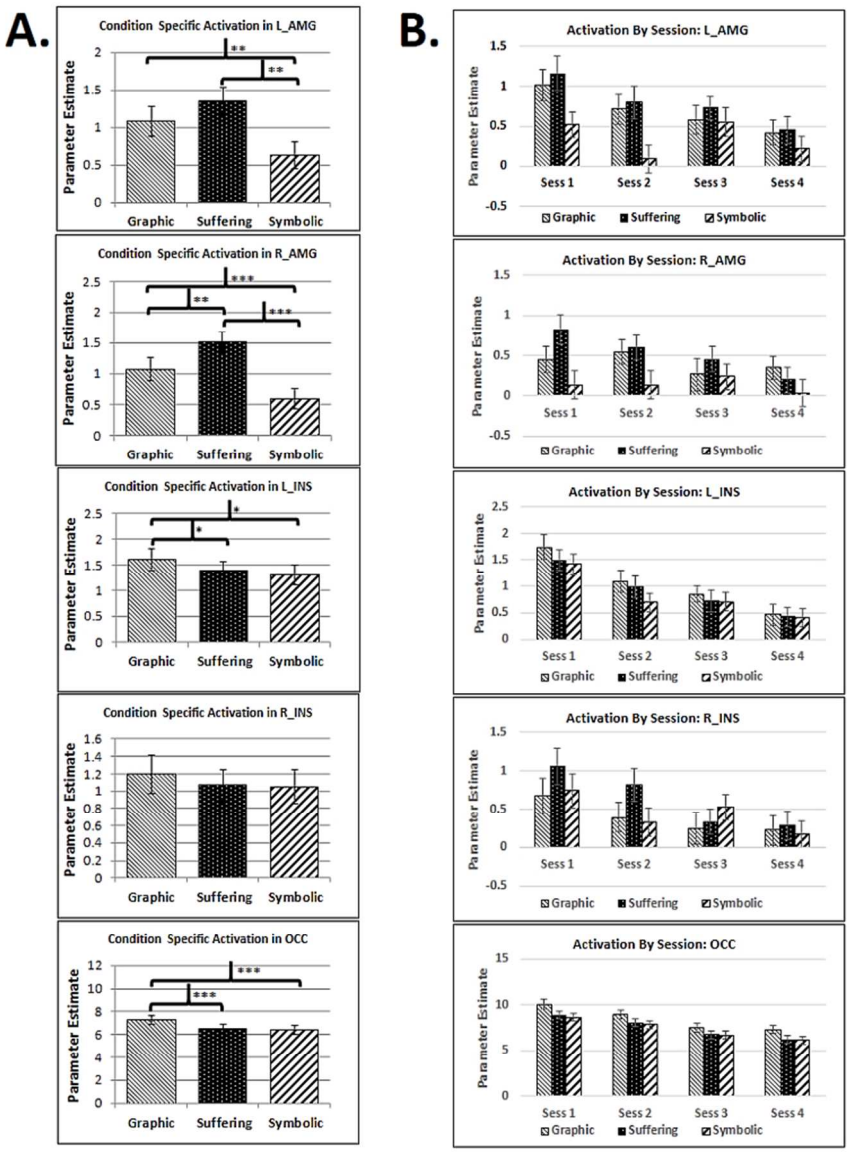

Fig. 4. (A)Results from region of interest (ROI) analyses. (B) Adaptation of BOLD signal in ROI's across four functional scanning runs. L_AMG = left amygdala {XYXmni = -26, -2, -17} , R_AMG = right amygdala {XYXmni = 23, 7, -17}, L_INS = left insula { XYXmni = -30, 30, 4}, R_INS = right insula { XYXmni = 28, 32, -8}, L_OCC = left occipital cortex{XYXmni = -26, -94, 4}, OCC = occipital cortex{XYXmni = -26, -94, 4; XYXmni = 24, -90, -6}, * = significant $p < 0.05$ (within subjects one-tailed t-test), ** = significant $p < 0.05$, *** = significant $p < 0.001$ (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM).

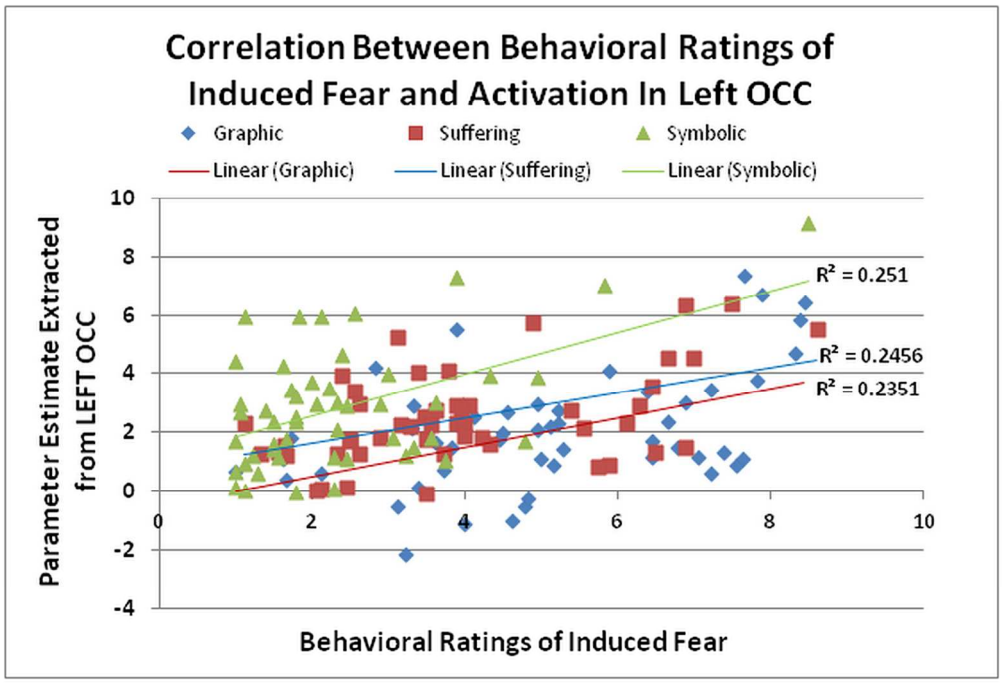

Figure 5. Correlation between BOLD signal in the visual association cortex (BA 18) and participant ratings different subtypes of HWL. The site of maximal correlation between the parameter estimates for the contrast (Graphic-Rest) and self-reported ratings of Graphic HWL stimuli was located at XYXmni = -19,-92,20. The site of maximal correlation between the parameter estimates for the contrast (Suffering-Rest) and self-reported ratings of Suffering HWL stimuli was located at XYXmni = -20,-88,12. The site of maximal correlation between parameter estimates for the contrast (Symbolic-Rest) and self-reported ratings of Symbolic HWL stimuli was located at XYXmni = -14,-92,12.

Image acquisition

All MRI data were collected on a 3T Siemens Trio system with a 12-element head coil housed at the McCausland Center for Neuroimaging at Palmetto Richland Hospital in Columbia SC. The fMRI (T_2* echo planar imaging) imaging sequence included the following parameters: 320 full brain volumes collected in each of the four 10-minute, 30-second sessions; 75° flip angle; time repetition (TR) = 1.95 s; time echo (TE) = 30 ms; in-plane resolution 3.30 × 3.30 mm; slice thickness = 3.0 mm (no gap); 36 axial slices collected in planes aligned parallel to the anterior commissure–posterior commissure line. To improve coregistration of images, all participants were scanned with a high-resolution T_1 MRI, which yielded a 1-mm isotropic image. This sequence had the following parameters: field of view (FOV) = 256 × 256 mm, 192 saggital slices, 9° flip angle, TR = 2250 ms, TE = 4.15 ms.

Data Pre-Processing

Functional MRI data were preprocessed using SPM (Wellcome Department of Cognitive Neurology, London), version 8. For the analysis of individual participant data, the following pre-statistics processing was applied: motion correction, coregistration, normalization and spatial smoothing. Motion correction employed SPM8's 4th Degree B-Spline interpolation. For coregistration, we first calculated the appropriate transform to bring each individual's mean EPI image into alignment with their structural image, and then applied this transform to the realigned EPI images. Normalization involved warping each individual's structural image onto the standard T1 weighted structural template and then applying this operation on the coregistered EPI images. As a final step in preprocessing, all EPI images were spatially smoothed using a Gaussian kernel of full width at half maximum 8.0 mm.

Data Analysis

At the first-level, we used SPM's general linear modeling approach to compute contrasts representing the main effect of each stimulus type (i.e., graphic, suffering, symbolic). The onsets and durations of each of the conditions of interest were modeled according to the block design described in the protocol. Functional data was modeled as a boxcar canonically convolved hemodynamic response function (duration 10 sec). For all group analyses reported below, we first generated a series contrast images for each individual participant (first-level models). Contrasts generated based on these first-level models were entered these into random effects models and/or regression models (using SPM's built in general linear model) in order to allow for meaningful population-level inference.

Supplementary Table 1.

Region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Graphic > Symbolic:					
*Lingual Gyrus	L	-16	-90	-8	11.98
*Primary Visual Cortex	R	22	-96	4	10.66
*Superior Parietal Lobule	L	-22	-70	40	6.07
*Superior Parietal Lobule	R	22	62	48	5.6
Inferior Parietal Lobule	L	-34	-38	44	4.69
Supramarginal Gyrus	R	60	-18	40	4.51
Amygdala	R	22	-4	-14	4.15
Precentral Gyrus	R	44	8	28	4.03
Inferior Parietal Lobule	L	-52	-28	36	3.96
Postcentral Gyrus	R	46	-30	44	3.76
Precentral Gyrus	L	-44	4	30	3.64
Amygdala	L	-20	-4	-12	3.6
Symbolic > Graphic:					
*Cuneus	R	4	-82	30	8.36
*Lingual Gyrus	R	10	-66	2	7.14
*Calcarine Gyrus	L	-8	-72	10	6.23
Supramarginal Gyrus	L	50	-34	22	4.63
Anterior Cingulate Gyrus	R	10	34	4	4.42
Middle Temporal Gyrus	R	54	-22	-6	4.40
Superior Temporal Gyrus	L	-52	-4	-12	4.27
IFG Pars Orbitalis	R	40	48	-4	3.74

L: left hemisphere; **R**: right hemisphere; **MNI :** Montreal Neurological Institute; **IFG** : Inferior frontal gyrus.
T-value: local maxima thresholded at $p < 0.001$, uncorrected, extent threshold $k = 10$
*values were significant after FWE correction, extent thresholding $k = 10$

Supp. Tbl. 1. Table of brain activations elicited by observation when comparing Graphic HWLs to Symbolic HWLs.

Supplementary Table 2.

Region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Suffering > Symbolic:					
*Fusiform Gyrus	R	42	-46	-18	8.99
*Post Middle Temporal Gyrus	R	54	-64	12	8.95
*Amygdala	R	20	-6	-10	7.85
*Precuneus	R	4	-58	38	7.03
*Hippocampus	L	-18	-8	-12	6.92
*Occipital Lobe	L	-46	-70	16	6.7
*IFG Pars Triangularis	R	42	18	24	5.89
*Hippocampus	R	18	-32	0	5.31
Ant. Middle Temporal Gyrus	R	58	0	-16	4.36
Orbital Frontal Gyrus	L	-2	56	-12	4.22
IFG Pars Triangularis	R	50	38	14	4.19
Cuneus	R	14	-95	14	3.96
Symbolic > Suffering:					
*Lingual Gyrus	L	-24	-58	-14	6.97
Lingual Gyrus	R	24	-58	-10	5.12
IFG Pars Triangularis	L	-38	42	10	4.78
Occipital Lobe	L	-30	-88	16	4.77
Anterior Cingulate	R	10	36	14	4.16
Superior Frontal Gyrus	R	22	50	10	3.70

L: left hemisphere; **R**: right hemisphere; **MNI :** Montreal Neurological Institute: **Ant.** : Anterior; **Post.** : Posterior; **IFG** : Inferior frontal gyrus.
T-value: local maxima thresholded at $p < 0.001$, uncorrected, extent threshold $k = 10$
*values were significant after FWE correction, extent thresholding $k = 10$

Supp. Tbl. 2. Table of brain activations elicited by observation when comparing Suffering HWLs to Symbolic HWLs.

Supplementary Table 3.

Region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Suffering > Graphic:					
*Post Middle Temporal Gyrus	R	50	-46	12	8.55
*Precuneus	R	4	-60	38	7.48
*Ant. Middle Temporal Gyrus	L	-54	-6	-12	6.87
*Ant. Middle Temporal Gyrus	R	56	-2	-16	6.42
*Post Middle Temporal Gyrus	L	-50	-50	12	6.39
*Orbital Frontal Gyrus	R	4	48	-12	6.20
*Lingual Gyrus	L	-12	-52	0	5.88
*Lingual Gyrus	R	12	-54	2	5.87
*Fusiform Gyrus	L	40	-45	-15	5.59
*Ant. Superior Temp. Gyrus	R	38	20	-28	5.43
*IFG Pars Triangularis	R	52	34	6	5.32
*Ant. Superior Temp. Gyrus	L	-46	10	-20	5.13
Hippocampus	R	28	-8	-14	4.77
Hippocampus	L	-20	-10	-14	4.24
Supplementary Motor Area	L	-2	-24	66	3.66
Graphic > Suffering:					
*Occipital Lobe	L	-30	-86	16	11.29
*Occipital Lobe	R	34	-82	12	10.67
*Fusiform Gyrus	L	-26	-56	-14	10.29
*Fusiform Gyrus	R	26	-56	-12	8.11
*Superior Parietal Lobe	R	26	-66	54	7.91
*Superior Parietal Lobe	L	-24	-74	36	7.24
*Inferior Temporal Gyrus	R	50	-56	-8	6.68
IFG Pars Opercularis	L	-46	2	30	5.03
Inferior Parietal Lobe	L	-40	-40	44	4.36
Middle Frontal Gyrus	L	-44	42	14	3.84
Supramarginal Gyrus	R	44	-32	44	3.77
Supramarginal Gyrus	R	50	-24	44	3.61

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute; **Ant.** : Anterior; **Post.** : Posterior; **IFG** : Inferior frontal gyrus.
T-value: local maxima thresholded at $p < 0.001$, uncorrected, extent threshold $k = 10$
*values were significant after FWE correction, extent thresholding $k = 10$

Supp. Tbl. 3. Table of brain activations elicited by observation when comparing Graphic HWLs to Suffering HWLs.

We confirm our compliance with the following STROBE statement recommendations for reporting cross-sectional studies.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a

		meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Neural biomarkers for assessing different types of imagery in pictorial health warning labels for cigarette packaging: A cross-sectional study.

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Neural biomarkers for assessing different types of imagery in pictorial health warning labels for cigarette packaging: A cross-sectional study.

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Abstract

Objective Countries around the world have increasingly adopted pictorial health warning labels (HWLs) for tobacco packages to warn consumers about smoking-related risks. Research on how pictorial HWLs work has primarily analyzed self-reported responses to HWLs; studies at the neural level comparing the brain's response to different types of HWLs may provide an important complement to prior studies, especially if self-reported responses are systematically biased. In this study we characterize the brain's response to three types of pictorial HWLs for which prior self-report studies indicated different levels of efficacy.

Methods Current smokers rated pictorial HWLs and then observed the same HLWs during functional magnetic resonance (fMRI) scanning. Fifty 18- to 50-year-old current adult smokers who were free from neurological disorders were recruited from the general population and participated in the study. Demographics, smoking-related behaviors, and self-reported ratings of pictorial HWL stimuli were obtained prior to scanning. Brain responses to HWLs were assessed using fMRI, focusing on *a priori* regions of interest.

Results Pictorial HWL stimuli elicited activation in a broad network of brain areas associated and visual processing and emotion. Participants who rated the stimuli as more emotionally arousing also showed greater neural responses at these sites.

Conclusions Self-reported ratings of pictorial HWLs are correlated with neural responses in brain areas associated with visual and emotional processing. Study results cross-validate self-reported ratings of pictorial HWLs and provide insights into how pictorial HWLs are processed.

Strengths and limitations of this study

- This is the first study to explore the relationship between self-reported ratings of pictorial HWLs and neural responses to pictorial HWLs in a large sample (N = 50) of current adult smokers.
- This paper demonstrates the amygdala is maximally activated by pictorial HWLs that depict human suffering, followed by images that depict graphic effects of smoking, followed by symbolic images of the negative consequences of smoking.
- This paper demonstrates that neural responses to pictorial HWLs attenuate with repeated exposure in most brain regions, but that this response is different in the amygdala.
- Further research is required in order to determine i) exactly why pictorial HWLs depicting human suffering elicited such robust responses in the amygdala and ii) whether differential adaptation to symbolic stimuli is relevant to the creation of optimal HWLs.

INTRODUCTION

According to the World Health Organization, smoking remains the leading cause of preventable death in the Western world.(1, 2) Smoking increases the risk of many non-communicable diseases both in smokers and in those who breathe second hand smoke.(3) To help prevent tobacco use and its consequences, the World Health Organization Framework Convention on Tobacco Control (WHO FCTC) has recommended including prominent, pictorial health warning labels (HWLs) on tobacco packaging to communicate the adverse effects of smoking to consumers and to discourage smoking.(2) Experimental and observational research indicate that HWLs with pictorial imagery are *more* effective than text-only HWLs in both promoting smoking cessation and preventing the initiation of smoking behavior.(4-7) A key advantage of pictorial HWLs is likely due to their ability to elicit stronger emotional responses than text-only HWLs.(8)

The increasing adoption of pictorial HWLs around the world has created a critical need for research designed to: i) evaluate the relative effectiveness of different types of HWL content; and ii) explain why some HWL content appears more effective than other content. Such research should guide the selection of HWL content, including the rotation of new HWL content over time. Some experimental research has found the self-reported effectiveness of pictorial HWLs is highest when it contains graphic images that depict the physical effects of smoking, followed by imagery of personal suffering (usually including a face), and finally by symbolic representations of smoking effects that use abstract imagery or symbols to represent risk.(9-12) These findings are consistent with some observational studies indicating that graphic depictions of smoking consequences work best.(13, 14)

The *primary goal* of the current experiment was to explicitly map neural responses to HWLs that contain three different subtypes of imagery that are frequently used in tobacco control communications, including HWLs on cigarette packaging: graphic representation of physical consequences of smoking; personal suffering from smoking-related consequences; and symbolic representations of risk. Given the visual and emotional nature of pictorial HWLs, we formulated a set of *a priori regions of interest* (ROIs) that we expected to respond to participants' observations of HWLs, including the amygdala, insula and visual cortex. Converging evidence from numerous neuroscientific investigations confirms a prominent role for the *amygdala* in emotional processing in a number of sensory modalities.(15-19) The amygdala plays a particularly important role in the processing of visual stimuli related to threat and fear.(20-22) We expected that amygdala responses would be driven by our stimuli to the extent that they elicited arousal, fear and perceived threat (e.g., graphic HWL vs. symbolic HWL). We also expected pictorial HWLs to elicit robust activity in the *insula*. This area has been linked to the experience of disgust, and strongly responds to pictures of mutilation and contamination.(23-26) Finally, based on a prior investigations of the neural response to emotional pictures, we expected the *visual association cortex* to be robustly activated by the presentation of pictorial HWLs.(27-29) We expected all three subtypes of HWLs to elicit a significant response (relative to rest) in this subset of *a priori* ROIs.

Our *secondary goal* was to examine the relationship between self-report data indicating that HWLs that use graphic imagery are more effective than HWLs depicting human suffering, which were in turn more effective than symbolic HWLs. We hypothesized that the neural response in our *a priori* ROIs would differentiate between our three types of HWL (graphic > suffering > symbolic), and that participants who rated pictorial HWL stimuli as more

emotionally arousing exhibit heightened activity in these areas. In order to examine these questions, 50 current adult smokers self-reported emotional arousal of HWLs of each pictorial subtype and subsequently observed the same stimuli while their brain activity was measured using fMRI.

METHOD

Participants

Fifty adult smokers between the ages of 18 and 50 (24 females, Mean Age = 27.57) took part in this study. Participants were recruited from the general public, via fliers posted in public locations around the University of South Carolina (USC) and local newspapers. All participants were neurologically healthy smokers with normal or corrected to normal vision. Following initial phone and online screening to confirm qualification for participation, all subjects reported to the McCausland Center and provided informed consent prior to MRI scanning. Following completion of the study protocol, participants were paid $100 for transportation costs related to participation in the study. The experiment was performed according to the guidelines of the Declaration of Helsinki and approved by the IRB at USC.

Pictorial HWL Stimuli

A total of 57 pictorial HWLs were used, with images drawn primarily from, based on, or considered for actual HWLs implemented in different countries (**Supplementary Figure 1**), including prior HWL research that has relied on self-reported responses to HWLs to determine the efficacy of different content.(6, 30, 31) Nineteen pictorial HWLs were developed for each of three pictorial styles that were matched on textual and topical content: 1) Graphic health effect - vivid depiction of physical effects of smoking on the body; 2) Human suffering - depiction of personal experience which shows the face and could include the physical, social or emotional

impact of smoking-related harm and; 3) symbolic – representation of health risks using abstract imagery or symbols. Prior HWL research indicates that adult smokers and adolescents discriminate between these three general categories of HWL imagery. (10, 32-36) The textual content to accompany pictorial elements involved short, factual statements based on HWLs that countries have implemented or that have been used in prior research.(9) HWL topics addressed 13 different health issues (i.e., addiction, death, emphysema, gangrene, heart disease, lung cancer, mouth cancer, pregnancy, breast cancer, second-hand smoke, strokes, throat cancer, and blindness), with some topics (emphysema, death, heart disease, lung cancer, mouth cancer, stroke) having two sets of three HWLs on the same health topic but with one of each different pictorial style (graphic, suffering, symbolic). Textual elements were matched across all three HWL subtypes. Importantly, the mean luminance values for pictorial HWL s did not differ between subtypes (all p's > 0.18), nor did the overall color (as measured by Red, Green or Blue color values) (all p's > 0.11).

Study Procedures

Demographic Data

All participants were asked standard questions regarding their age, gender, income, ethnicity, and current and past use of cigarettes (**Supplementary Table 1)**.

Self-reported Responses to HWLs

Prior to attending the laboratory session, each participant completed a short survey and rated all 57 HWLs, which were presented online and in random order. The primary reason for collecting the self-report ratings before the fMRI experiment was to minimize respondent

burden, as the fMRI protocol lasted an hour. We gauged this as a greater concern than familiarization (which could attenuate subsequent BOLD response), especially as smokers are usually exposed to HWLs many times every day. Negative emotional arousal was assessed by asking participants to rate the HWL on how much it made them afraid ("How much does this warning make you feel afraid?"). As in prior research, (9, 12) participants were also queried concerning ad effectiveness ("How effective is this warning?). For both questions, participants responded with a rating of 1 to 9, with verbal anchors at either end of the rating scale (i.e., 1 = not at all, 9 = extremely).

Smoking Status Screening

To confirm smoking status, carbon monoxide (CO) levels were measured in all participants immediately prior to scanning using a piCO+ Smokerlyzer (Bedfont Scientific, Harrietsham, England). All participants also provided saliva samples immediately prior to scanning to assess cotinine (nicotine metabolite) using liquid chromatography with Tandem Mass Spectrometry (LC-MS/MS). These assays confirmed self-reported smoking status for all participants. Participants also reported the time since last cigarette, the number of days they smoked in the last 30 days, and the average number of cigarettes they smoked per day during that time (**Supplementary Table 1**).

Neural Response to HWLs

During 50 minutes of MRI scanning, each participant completed a single, high resolution structural scan, as well as four functional MRI task runs. Each functional run was 10 minutes and 24 seconds in duration. During the entire scanning session of four runs, each of the 57 images (19 graphic images, 19 suffering images and 19 symbolic images) was presented a total

of 10 times each. These images were presented using a block design format. Each block of stimuli was 15 seconds in duration and consisted of the serial presentation of 5 images from the relevant condition (or fixation cross for Rest), separated by 1 second of fixation. A total of 40 blocks (10 graphic images, 10 suffering images, 10 symbolic images and 10 Rest) were presented during each of four functional runs, for a total of 150 HWLs per functional run (50 in each category). The 150 images within a given functional run were randomly chosen from a pool of 600 images created at the beginning of the scanning session. This pool of 600 images consisted of 10 of each individual HWL (10*19*3 = 570), with the remaining 30 being randomly chosen (10 pseudo-random choices from each category-the constraint being that they all had to be different, i.e. no repeats within this subset) (**Figure 1**) The order of presentation of the blocks within a given functional run was chosen from one of eight pseudo-randomly generated trial orders. These orders were constrained such that i) each condition was equally likely to follow any other condition within a certain functional run; and ii) blocks of the same trial type never occurred more than three times in a row. Each of the four functional runs was identical in duration and content with the exception of the random assignment of images from each condition to its corresponding block. Importantly, the total time (and thus total number of brain volumes recorded) spent showing blocks of each picture type was identical to the total time spent showing Rest blocks.

In order to ensure that participants paid attention to the visual stimuli, we employed a 1-back picture recognition task. Participants were instructed to press a button when the same picture appeared twice in a row. Each functional run contained either 5 or 6 repeated pictures which required the participant to press a button. Placement of repeats was randomized prior to each run using Presentation's built in randomization features.

fMRI Methods

Image Acquisition

All MRI data were collected on a 3T Siemens Trio system with a 12-element head coil. The fMRI (T_2* echo planar imaging) imaging sequence included the following parameters: 320 full brain volumes collected in each of the four 10-minute, 24-second runs; 75° flip angle; time repetition (TR) = 1.95 s; time echo (TE) = 30 ms.; in-plane resolution 3.30 × 3.30 mm; slice thickness = 3.0 mm (no gap); 36 axial slices collected in planes aligned parallel to the anterior commissure–posterior commissure line. To improve coregistration of images, all participants were scanned with a high-resolution T_1 MRI, which yielded a 1-mm isotropic image. This sequence had the following parameters: field of view (FOV) = 256 × 256 mm, 192 saggital slices, 9° flip angle, TR = 2250 ms., TE = 4.15 ms.

Data preprocessing and Modelling

All fMRI data were preprocessed and analyzed using SPM8 (Wellcome Department of Cognitive Neurology, London). Standard preprocessing procedures included image realignment (4^{th} Degree B-Spline Interpolation), coregistration (Mean EPI aligned with T1 then parameters applied to all EPIs), normalization and spatial smoothing (Gaussian Kernel FWHM 8mm). The onsets and durations of each of the conditions of interest were modeled according to the block design described in the protocol. For our primary analysis, functional data across the four runs was modeled as a boxcar canonically convolved hemodynamic response function (duration 10 seconds). For results regarding between-run differences (i.e. neural adaptation), condition-specific activation within each functional run was modeled as a separate set of events. For all group analyses reported below, we first generated a series contrast images for each individual participant (first-level models) and then entered these into random-effects models and/or

regression models (using SPM's built in general linear model) in order to allow for meaningful population-level inference. First eigen-variates were extracted from second-level models (for each ROI/condition/run) using the VOI toolbox in SPM 8.(37) For the multiple regression analysis between self-reported ratings and neural responses reported below, means for neural responses were calculated at the HWL level (mean values were calculated for each participant for the neural response in each ROI and for each HWL subtype). The resulting parameter estimates were used as the primary dependent variables in the statistical models reported below (i.e. ANOVA and regression analyses).

RESULTS

Behavioral Performance

Population Variables

Our Participants in the current study were equally spilt with respect to gender (52% Male, 48% Female) and predominantly white (74%, 24% African American, 2% other). The majority of participants (55%) had at least some post-high school education, and were low-income. At the time of scanning, the group's CO levels were 18.74 ppm and cotinine was measured at 207.48 ng/mm confirming that all participants were active smokers. Furthermore, the average participant smoked 18.74 cigarettes per day, and reported having smoked on 28.32 out of the previous 30 days.

Self-reported Ratings of HWLs

Differences in self-reported emotional arousal across the three stimulus types (graphic, suffering, symbolic) was assessed using one-way within subjects ANOVA, $F(1.44,70.53) = 121.01$, $p < 0.001$. A one-way within subjects ANOVA using perceived effectiveness as a dependent variable and stimulus-type (graphic, suffering, symbolic) as the dependent variable

was also significant, $F(1.54,75.27) = 133.27$, $p < 0.001$. For both ANOVAS, post-hoc pair-wise comparisons revealed significant differences between ratings of graphic and suffering stimuli, as well as between ratings of suffering and symbolic stimuli (all p's < 0.01).

Responses to the emotional arousal and perceived effectiveness questions were highly correlated for the graphic ($r(49) = .87$), suffering ($r(49) = .90$) and symbolic ($r(49) = .90$) stimuli. Because ratings of emotionality were the most relevant for interpretation of our results, we focus on those scores in our analysis section. When the same analyses were conducted using perceived effectiveness, we obtained a similar pattern of results (i.e., graphic > suffering > symbolic). (**Figure 2**)

fMRI One-back Task:

One-back task performance data was collected from a total of 176 out of 200 possible fMRI scanning runs (50 participants, with 4 runs per person). Data from 24 of the runs was lost due to experimenter error. We did not exclude the imaging data from these participants as we did monitor the participants' error rates online and ensure they were paying attention (they were just not recorded). A one-way ANOVA using *error rate* as the dependent variable and run as the factor was not significant, $F(3,162) = 1.003$, $p = 0.393$. Moreover, post-hoc comparison failed to reveal any significant differences between error rates in any two runs (all p-values > 0.33).

fMRI Response

Primary fMRI Outcomes

Main Effects of HWL Type

In order to isolate cortical networks activated by the presentation of each type of pictorial HWL, we computed a series of contrasts designed to test for the main effects of each of the three stimulus types (graphic, suffering, and symbolic). Specifically, we computed the following

contrasts: graphic-Rest, suffering-Rest and symbolic-Rest (thresholded at $p < 0.05$ and corrected for family-wise error [FEW]). Observation of pictorial HWL stimuli elicited a significant neural response in a broad network of brain areas including our *a priori* ROIs (the amygdala, insula, and visual association cortex) as well as a number of other brain areas including the frontal gyrus (inferior, middle, medial, and superior aspects), temporal gyrus (middle and superior), parietal lobe (inferior), supplementary motor area, parahippocampal gyrus, and thalamus. The results of this analysis are listed in **Table 1** and displayed graphically in **Figure 3**.

Comparison of HWL-elicited Activation in a priori ROIs

We performed additional analyses in order to identify brain areas that responded maximally to graphic HWLs, less to suffering HWLs and least to symbolic HWLs. Accordingly, we performed ROI analyses on our *a priori* ROIs including the amygdala, insula and secondary visual cortex. ROIs within the visual association cortex, amygdala and insula were created based on peak activations observed in the contrast comparing the brain's response to all conditions to rest ([graphic + suffering + symbolic] – Rest).(37) All ROIs were centered at the site of peak activation within a given ROI and were spherical in nature (r = 4 mm). A series of one-way within-subjects ANOVAs were used to evaluate neural responses patterns (for graphic, suffering and symbolic stimuli) within our ROIs. These ANOVAs were significant in the left amygdala, $F(2,98) = 14.59$, $p < 0.001$, right amygdala, $F(2,98) = 21.60$, $p < 0.001$, left insula, $F(2,98) = 4.42$, $p < 0.05$, and visual association cortex, $F(2,98) = 22.69$, $p < 0.001$. As with the behavioral data, we conducted post-hoc pairwise comparisons (all significant results were $p < 0.05$, Bonferroni corrected). In the left amygdala we observed a significant difference between responses in the graphic and symbolic conditions, as well as in the suffering and symbolic conditions. In the right amygdala all pair-wise comparisons were significant. In the left

amygdala and the visual association cortex, responses to graphic and symbolic stimuli were significantly different, as were responses to graphic and suffering stimuli. The results of these analyses are shown graphically in **Figure 4, A.** We also conducted whole-brain analyses for the following direct comparisons between conditions: graphic > symbolic : symbolic > graphic (**Supplementary Table 2**), suffering > symbolic : symbolic < suffering, (**Supplementary Table 3**) and suffering > graphic : graphic > suffering (**Supplementary Table 4**).

Secondary fMRI Outcomes

Correlation Between Self-Reported Ratings and Neural Response

We ran a series of targeted correlations to determine whether there was a relationship between individual ratings of pictorial HWLs of specific subtypes and the BOLD signal elicited by their presentation. For the graphic stimuli, we conducted an SPM multiple regression analysis using individual contrast images for the graphic-Rest condition as the dependent variable and mean self-reported arousal ratings for the graphic HWLs as the independent variable (thresholded at $p < 0.001$, 5 voxel extent). Similar regression analyses were conducted to examine the correlation between HWL ratings and BOLD signal in the suffering and symbolic conditions. In all three analyses, activation in the right visual association cortex (XYZ_{mni} = -18, -92, 20, XYZ_{mni} = -20, -88, 12, and XYZ_{mni} = -14, -92,12 respectively) was positively correlated with mean ratings of the pictorial HWLs (all r(49)'s > .48) (**Figure 5**). For graphic and suffering HWLs additional positive correlations were found at sites in the right precentral gyrus (XYZ_{mni} = 44,4,40), r(49) = .45 and r(49) = .42 respectively. For symbolic HWLs there was an additional positive correlation between HWL ratings and activation in the left inferior frontal gyrus (XYZ_{mni} = -52,16,30), r(49) = .37).

Exploratory Analysis of BOLD Signal Adaptation

In addition to examining the main effects of stimulus type, we also conducted a series of 3 (Stimulus) x 4 (Session) repeated measures ANOVAs (one for each ROI) in order to explore possible BOLD signal adaptation to our three stimuli types across the four fMRI runs. The main effect of run was significant for the left insula, $F(3,138) = 11.40$, $p < 0.001$, right insula $F(3,138) = 3.19$, $p < 0.05$, and visual association cortex, $F(3,138) = 15.43$, $p < 0.001$, and nearly significant in the left amygdala, $F(3,138) = 2.66$, $p = 0.07$. There was a significant interaction between Stimulus and Run in both the left amygdala, $F(6,276) = 2.28$, $p < 0.05$, and right amygdala, $F(6,276) = 2.15$, $p < 0.05$. These results are shown split by run (in order to visualize adaptation) in **Figure 4, B**.

DISCUSSION

Self-reported Ratings of Pictorial HWLs

Results from the current study were generally consistent with prior research using self-reported responses to HWL stimuli. This research consistently indicates that smokers report stronger responses to HWLs with graphic imagery than to symbolic imagery. (10, 11, 32, 34, 35, 38) Results suggesting the greater impact of imagery of suffering than graphic imagery are not necessarily inconsistent with this research. Indeed, a number of the suffering images included graphic elements, and HWLs that combine the two may be may be most effective. (32) Nevertheless, as for self-report research, future fMRI research is needed to determine whether neural responses predict meaning behavioral change (i.e., quitting smoking) or perceptual change (e.g., better understanding of risks, particularly among youth). In general, however, this study suggests that fMRI and self-report produce similar results. One possible concern with the present results is that we did not confirm our specific sample of participants considered each pictorial

HWL to belong to one category or another. Future research may consider asking participants to sort pictorial HWLs into categories to address this concern.

Main Effects of HWL Type

The primary goal of the current experiment was to assess neural responses to the presentation of different types of pictorial HWLs that governments have considered for implementation. In general, observation of pictorial HWLs activated large-scale neural networks including the hippocampus, fusiform gyrus, precentral gyrus, supplementary motor area, pars Triangularis, pars opercularis, pars orbitalis and fusiform gyrus. Based on prior literature mapping the brain's response to vivid graphic images, we expected all three types of HWLs to elicit activation in the amygdala, the insula and the visual association cortex. Our results are consistent with this literature in that all subtypes of pictorial HWLs used in the current study elicited activation at sites in all three of these areas.

Comparison of HWL-elicited Activation in *a priori* ROIs

Visual Association Cortex

We expected the intensity of BOLD signal in regions associated with visual and emotional processing to mirror self-reported ratings of the stimuli (i.e. graphic > suffering > symbolic). Results from our ROI analysis were partially consistent with this prediction. Activity in the right visual association cortex did scale in the same manner as self-reported ratings of the HWL stimuli. The more vivid/graphic nature of certain subtypes of pictorial HWLs may be responsible for the differences we observed in the visual cortex. Images in the graphic condition contained more gory/bloody elements than those in any of the other two conditions; the images in the suffering condition contained a moderate amount of these elements; and images in the symbolic condition contained the least of these elements. We speculate that these negatively

valenced elements, which were particularly arousing, may have increased signal in visual areas via afferent projections from the amygdala. It is well established that the amygdala, a key neural pathway for responses to graphic imagery, projects to both primary and secondary visual cortices.(39) It is particularly unlikely that heightened activation in the visual association cortex was caused by differences in low-level features of the images. Neither luminance nor color values for HWL stimuli were significantly different across the three HWL subtypes. Additionally, in at least one previous experiment examining the impact of arousing visual stimuli on visual cortex activity, differences in eye movements did not account for the observed patterns of activation.(28) Therefore it is unlikely that the effects we report were due to differential eye movements.

Amygdala

While responses in the visual association area and insula were consistent with self-reported ratings, activation patterns observed in amygdala were not. Unexpectedly, the amygdala was most robustly activated by suffering HWLs, followed by graphic HWLs, and finally symbolic HWLs. As noted in the introduction, the amygdala has been shown to be responsive to arousing stimuli, and fear-evoking stimuli robustly activate this brain structure. One possibility, then, is that the HWLs depicting personal suffering from smoking-related outcomes are effective at eliciting fear in current adult smokers. However, this is inconsistent with the self-reported data, which indicated that graphic HWLs elicited maximal fear responses. A more parsimonious explanation for this finding is that the relatively higher activation hyper-activation observed for HWLs with suffering imagery was due to the presence of human faces in the stimuli (all 19 suffering HWLs contained human faces). Lesion, single-cell and whole brain neuroimaging experiments are consistent with the idea that the amygdala is a key component of the face-

perception network.(18, 40-45) The amygdala may even process fearful facial stimuli in the absence of conscious processing.(46, 47) Hence, the inclusion of faces may be particularly important to maintaining arousal-inducing responses under conditions of repeated exposure, as is typically the case with HWLs. Indeed, recent evidence suggests that sustained responses to repeated presentation of emotional faces may be particularly dependent on the amygdala.(48) It is also important to note that some of the suffering images (4 of 19) portrayed visible body damage, and thus suffering imagery was not entirely distinct from graphic imagery used in the current experiment, and research based on self-reported ratings indicated that this combination produces the strongest ratings.(32) To better isolate any differential effects of these two image types and the interaction between them, future studies should use imagery that more clearly falls into one category, the other, or both. Another possible explanation for the increased relative amygdala activation observed in the suffering condition relates to stimulus salience (an index of stimulus salience). Studies have demonstrated a strong link between amygdala activation and stimulus salience.(49, 50)

While these results could have implications for the optimization of HWLs, further experiments are necessary to evaluate the predictive validity of fMRI. Future research should aim to separate out the effects of emotionality, salience and human faces by integrating additional conditions (such as neutral images with and without faces). Based on research demonstrating the that BOLD signal in the amygdala is a predictor of subsequent quitting behavior (51) (as is BOLD signal in the medial prefrontal cortex (52, 53)), future prospective studies should examine the extent to which amygdalar BOLD response to the three types of HWLs discussed in the current paper predict changes in smoking behavior or, among youth, perceptions about smoking-related risks. Little research has been conducted with youth before

they start smoking, and the strongest effects of HWLs may be due to enhancing aversion for smoking as opposed to changing the behaviors of addicted smokers.

Secondary fMRI Outcomes

Correlation Between Self-Reported Ratings and Neural Response

An important goal of the present study was to cross-validate self-reported ratings of pictorial HWLs and brain activity recorded during the observation of the same stimuli. This paper is the first to report such results for cigarette HWL stimuli. Regarding correlations between self-reported ratings of HWL stimuli and neural activity in our three *a priori* ROIs, only the visual cortex was significant (with the amygdala being nearly significant at p=0.07). We also report significant correlations between behavioral ratings and two additional areas, the junction of the right precentral and inferior frontal gyrus, and the left inferior frontal gyrus pars opercularis.

Visual Association Cortex

Our correlational data indicate that participants who rated pictorial HWL stimuli (within each category – as opposed to between categories) as more emotionally arousing showed higher activation of the visual association cortex when viewing the stimuli. This finding is consistent with previous reports demonstrating that activity in the visual cortex is particularly robust during the presentation of emotionally arousing visual stimuli, perhaps due to reentrant enhancement of V2 activity being driven by motivational processes that heighten input from the amygdala. (27, 29, 54)

Insula and Amygdala

Surprisingly, we did not observe a significant correlation between BOLD signal in the insula or amygdala and self-reported ratings of arousal. However, the correlation between BOLD

signal in the right amygdala and self-reported responses in both graphic (r(49) = .21, p = 0.07 one-tailed) and guffering (r(49) = .20, p = 0.08 one-tailed) conditions was nearly significant. This failure to reach statistical significance may be due to a number of reasons. One possibility is that the amygdala's response to the emotional stimuli was blunted by the inclusion of text in the HWLs used in the present study. This interpretation is consistent with a comprehensive meta-regression analysis of imaging studies on amygdala activation, which found that presence of language in the stimulus was associated with reduced amygdala activation (as well as greater left lateralization relative to baseline).(55) While the inclusion of text in graphic warning labels has traditionally been justified in terms of added information content (text adds information otherwise not present), it may also be important to examine possible emotional 'blunting' effects that its inclusion may have. Future brain imaging studies might explore this possibility by simultaneously monitoring brain activity and gaze behavior. A better understanding of the how people process graphical and textual elements of HWLs, and how attention to one or the other affects neural processing, particularly after repeated HWL exposure that simulates naturalistic exposure conditions, may help inform the design of future HWLs.

Junction of Right Precentral Gyrus and Inferior Frontal Gyrus

We also observed an unexpected correlation between self-report ratings and activity at the junction of the right precentral gyrus and inferior frontal gyrus (pars opercularis) for suffering HWLs only. Given the location of the activation in the RH (as opposed to the LH which is traditionally associated with such language functions), it is unlikely that heightened responses reflect increased reliance on language. This site is considered to be part of the human mirror neuron system (MNS) and thought to interact with the amygdala and insula when a link is established between the actions/emotions/intentions of others and our own actions.(56) One

possible explanation for this finding is that suffering stimuli may have been particularly effective at eliciting the types of interpersonal comparisons and or emotions (i.e. empathy) that individuals typically make when seeing the negative effects of their own behaviors in others.(53, 57-59) Another possible explanation for the significant correlation we observed between right IFG activity and self-reported ratings is that more emotionally arousing stimuli required greater emotion regulation on the part of the observer. This is consistent with studies reporting recruitment of the right IFG during tasks that require the inhibition of emotions. (60-62)

Inferior Frontal Gyrus, Pars Opercularis

Finally, we observed a significant relationship between activity in the left inferior frontal gyrus (BA 44) and self-report ratings of the symbolic stimuli. This area has traditionally been associated with language processing and is active during both overt (i.e. spoken) and covert (i.e. silent) speech.(63-67) It is not surprising that symbolic stimuli would utilize language processes. Stimuli of this subtype were the most abstract and likely evoked covert speech during the interpretation process. The involvement of language areas during HWL processing could be the topic of future experiments that assess verbalization during presentation of HWLs of all types. While it is reasonable to expect that activation of language areas during HWL processing (an indirect measure of covert verbalization) may be related to subsequent behavioral change, future studies will need to address this possibility.

Exploratory Analysis of BOLD Signal Adaptation

To the extent that HWL effectiveness depends on enduring emotional responses, neural adaptation to repeated exposure may be an important issue to consider. Our exploratory, post-hoc analysis of region-specific adaptation revealed that, in the majority of our ROIs, BOLD response decreased as a function of repeated exposure to all HWLs. Interestingly, we observed a

significant deviation from this pattern in the left and right amygdala. While activation associated with observation of graphic and suffering images was higher overall, it consistently decreased across the four runs, whereas activation patterns associated with observation of symbolic images was lower and less consistent (**Figure 4, B**). Hence, participants may not have adapted (neurally speaking) to repeated presentation of symbolic stimuli in the same way they adapted to images in the suffering and symbolic categories. The abstract nature of symbolic stimuli may have required additional exposures in order to more fully process their meaning, and this may account for the observed findings. These data should be interpreted cautiously, however, as repeated exposure to HWLs during three, 10-minute scanning runs is unlikely to accurately mimic repeated exposure to HWLs as in real-life, which is temporally spread out, situation specific, and associated with cravings and branding imagery that weakens HWL effects. Future research should more directly examine the relationship between the strength of brain activity elicited by specific subtypes of pictorial HWLs after repeated exposures to HWLs, including more naturalistic exposures that allow for adaptation and habituation.

Possible Implications for Public Health Policy

Understanding how the brain responds to HWLs can inform the optimal development of HWLs. For example, studies on smokers' neural responses to different types of anti-smoking ads has found that the strength of neural responses elicited by health messaging predicts subsequent individual-level behavioral change as well as the population-level efficacy of different types of ads responses to ads once they are aired in media campaigns.(53) While the current study does not report on behavioral change, future research should. Furthermore, if predictive validity of these methods is established, they could be used to assess the behavioral effects of other types of HWL content. The cost-effectiveness of fMRI compared to self-report

studies should also be assessed, particularly if they provide consistent results, as we have found here. Data regarding neural adaptation caused by repeated exposure to pictorial HWLs is could also be important in terms of informing the creation of HWLs designed for maximum long-lasting impact. Arguably, HWLs will only be effective to the extent that they continue to elicit responses from the consumer. Knowing whether or not consumers differentially adapt to different types of HWL content will allow for choice of HWLs that are most likely to discourage smoking.

Study Limitations

Understanding how the brain responds to HWLs can inform the optimal development of HWLs. For example, studies on smokers' neural responses to different types of anti-smoking ads has found that the strength of neural responses predicts subsequent individual-level cessation behavior(53) as well as population-level cessation attempts (i.e., volume of calls to quitlines) due to different types of ads once they are aired in media campaigns.(68) While the current study does not report on behavioral change, future research should. Furthermore, if the predictive validity of these methods is established, they could be used to evaluate the efficacy of a range of HWL content and presentation styles. The cost-effectiveness of fMRI compared to self-report studies should also be assessed, particularly if they provide consistent results, as we have found here. Data regarding neural adaptation caused by repeated exposure to pictorial HWLs could also be important in terms of informing the creation of HWLs designed for maximum long-lasting impact. HWLs are likely to be most effective if they elicit consumer responses over time. Indeed, the motivation to process messages changes over time, as does the motivation to quit smoking (69) and HWLs effects may become more potent as these motivations change. Knowing more about the process of adaptation to different types of HWL content,

including potential differences in the processes of adaptation across diverse groups, may help with designing HWLs that are most likely to discourage smoking.

General Conclusion

The present study examined adult smokers' self-reported and neural responses to three different types of pictorial HWL stimuli that governments commonly use on cigarette packaging. Pictorial HWLs elicited robust responses in an extensive network of brain sites including those associated with image interpretation (visual association cortex) and emotion (amygdala and insula). Moreover, activation in visual, premotor, inferior frontal and, to a lesser extent, the insular areas, varied in a manner consistent with self-reported ratings of the stimuli. We report a robust relationship between self-reported ratings of arousal and neural responses, which is important considering that self-reported data can be subject to bias. Our exploratory, post hoc analysis of BOLD signal attenuation across scanning runs revealed differences in the patterns of neural adaptation for different types of HWLs that may be relevant to the optimization of future HWLs. Gaining a better grasp of the relationship between self-reported ratings of HWLs, neural responses elicited by HWLs, and the effectiveness of HWLs should be an important goal of future research.

Figure Legends

Fig. 1. graphical representation of the construction of each functional run. All stimuli types (graphic, suffering, and symbolic) were presented in block format. Each block consisted of the presentation of five pseudo-randomly selected stimuli of the appropriate type presented for 2 seconds each, and separated by 1 second of fixation. Block order was pseudo-randomized for each functional run.

Fig. 2. Behavioral effectiveness ratings of HWLs. All participants rated all HWLs prior to fMRI scanning by responding to the question: **"**How much does this warning make you feel afraid?". *** = significant $p < 0.001$ (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM).

Fig. 3. Main effects of HWLs on BOLD signal (graphic, suffering, symbolic) on BOLD signal. All results are thresholded at $p < 0.05$ and corrected for family-wise error (FWE). Results are overlaid on a standard inflated brain (cortex_20484.surf.gii) for illustration purposes.

Fig. 4. (A) Results from ROI analyses. **(B)** Adaptation of BOLD signal in ROIs across four functional scanning runs. L_AMG = left amygdala {XYX_{mni} = -26, -2, -17} , R_AMG = right amygdala {XYX_{mni} = 23, 7, -17}, L_INS = left insula { XYX_{mni} = -30, 30, 4}, R_INS = right insula { XYX_{mni} = 28, 32, -8}, L_OCC = left occipital cortex{XYX_{mni} = -26, -94, 4}, OCC = occipital cortex{XYX_{mni} = -26, -94, 4; XYX_{mni} = 24, -90, -6}, * = significant $p < 0.05$ (within subjects one-tailed t-test), ** = significant $p < 0.05$, *** = significant $p < 0.001$ (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM).

Fig. 5. Correlation between BOLD signal in the visual association cortex (BA 18) and participant self-reported ratings of different subtypes of HWL. The site of maximal correlation between the parameter estimates for the contrast (graphic-Rest) and self-reported ratings of graphic HWL stimuli was located at {XYX_{mni} = -19,-92,20}. The site of maximal correlation between the parameter estimates for the contrast (suffering-Rest) and self-reported ratings of suffering HWL stimuli was located at {XYX_{mni} = -20,-88,12}. The site of maximal correlation between parameter estimates for the contrast (symbolic-Rest) and self-reported ratings of symbolic HWL stimuli was located at {XYX_{mni} = -14,-92,12}.

Table 1.

region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
ALL - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**21.62**
Fusiform Gyrus	R	42	-80	-10	19.48
Calcarine	R	12	-94	0	19.02
Hippocampus	R	20	-30	0	15.8
Hippocampus	L	-22	-30	-2	13.73
IFG Pars Triangularis	L	-52	24	30	9.87
Precentral Gyrus	L	-46	-4	52	9.71
Precentral Gyrus	L	-42	8	32	9.26
SMA	L	-6	8	56	8.99
SMA	R	6	10	52	8.53
IFG Pars Triangularis	R	48	24	26	8.67
IFG Pars Opercularis	R	54	22	32	8.66
Middle Frontal Gyrus	R	50	36	24	8.64
Insula	**L**	**-30**	**28**	**2**	**8.39**
IFG Pars Orbitalis	L	-34	30	-8	8.17
IFG Pars Orbitalis	L	-40	26	-12	7.81
Amygdala	**R**	**20**	**-6**	**-14**	**7.33**
Amygdala	**L**	**-22**	**-4**	**-14**	**6.47**
IFG Pars Orbitalis	R	28	30	-10	6.12
Insula	**R**	**32**	**30**	**2**	**5.57**
Fusiform Gyrus	L	-32	-32	-16	6.02
Parahippocampal Gyrus	L	-14	-28	-16	5.13
graphic - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.86**
Declive	L	-38	-70	-10	19.05
Fusiform Gyrus	R	42	-80	-10	18.41
Hippocampus	L	-22	-30	-2	11.35
Hippocampus	R	22	-30	0	13.19
Precentral Gyrus	L	-46	-4	48	9.42
Precentral Gyrus	L	-50	6	38	8.68
Precentral Gyrus	L	-42	6	32	8.47
SMA	L	-6	6	58	8.54
SMA	R	6	10	52	7.87
Precentral Gyrus	R	46	8	34	8.36

Middle Frontal Gyrus	R	50	36	24	8.31
IFG Pars Opercularis	R	54	22	30	7.91
Insula	**L**	**-30**	**30**	**-4**	**7.46**
Parahippocampal Gyrus	R	36	-6	-26	6.54
Amygdala	**L**	**-22**	**-2**	**-16**	**6.38**
Amygdala	**R**	**22**	**-4**	**-14**	**6.1**
Parahippocampal Gyrus	L	-30	-34	-16	5.94
IFG Pars Orbitalis	R	28	30	-10	5.69
Middle Temporal Gyrus	L	-54	-46	8	5.42
suffering - Rest:					
Fusiform Gyrus	R	42	-80	-10	19.19
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.1**
Occipital Lobe (Middle)	**L**	**-26**	**-96**	**8**	**18.46**
Hippocampus	R	24	-28	-2	15.59
Hippocampus	L	-22	-28	-4	14.41
Amygdala	**R**	**20**	**-6**	**-14**	**9.36**
IFG Pars Triangularis	R	52	30	26	9.05
IFG Pars Opercularis	R	46	14	32	8.54
IFG Pars Opercularis	R	52	20	34	7.88
Insula	**L**	**-30**	**28**	**0**	**8.65**
Inferior Frontal Gyrus	L	-36	20	-18	5.25
Precentral Gyrus	L	-46	-4	48	8.48
Precentral Gyrus	L	-40	8	32	8.42
IFG Pars Triangularis	L	-44	18	26	7.72
SMA	R	6	10	52	8.14
Amygdala	**L**	**-20**	**-6**	**-14**	**7.71**
Superior Temporal Gyrus	L	-52	-52	10	7.4
Insula	**R**	**30**	**32**	**-8**	**6.31**
Inferior Parietal Lobule	L	-48	-26	52	5.56
Superior Temporal Gyrus	R	48	-40	10	5.4
symbolic - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.56**
Cuneus	**L**	**-18**	**-100**	**6**	**18.61**
Lingual Gyrus	**R**	**12**	**-94**	**0**	**17.98**
Hippocampus	R	22	-28	-2	14.14
Hippocampus	L	-22	-30	-2	11.36
IFG Pars Triangularis	L	-50	22	30	8.92
IFG Pars Opercularis	L	-42	10	30	8.57
Precentral Gyrus	L	-46	-4	48	8.5
SMA	L	-4	8	56	8.77
SMA	R	6	12	52	8.72
IFG Pars Opercularis	R	54	22	32	7.68

Middle Frontal Gyrus	R	50	36	24	7.59
Precentral Gyrus	R	46	12	32	6.76
Insula	**L**	**-30**	**28**	**0**	**7.28**
IFG Pars Orbitalis	L	-36	28	-10	7.2
Inferior Parietal Lobule	L	-46	-38	54	6.19
Inferior Parietal Lobule	L	-48	-28	52	5.32
Insula	**R**	**32**	**30**	**2**	**5.2**

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute
T-value: local maxima thresholded at $p < 0.05$ FWE corrected, extent threshold $k = 10$
a-priori ROIs indicated in **BOLD.**

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Competing Interests Statement

The authors and coauthors have no competing interests to declare.

Contributorship Statement

Roger D. Newman-Norlund helped design the experiment, collected MRI data, analyze behavioral and MRI data, conducted statistical analyses and drafted the paper. James F. Thrasher oversaw the experiment, helped design the experiment, and helped draft the paper. Johann Fridriksson helped design the experiment, recruited participants, collected behavioral and MRI data and revised the draft paper. William Brixius helped collect MRI data and revised the draft paper. Brett E. Froeliger, David Hammond and Michael K. Cummings helped design the experiment and draft the paper.

Data Sharing Statement

All data collected in this experiment is located on the hard drive of the corresponding author, R.D.N. Data analysis is ongoing and the data may still be used for additional papers. After all planned papers have been submitted, the data may be made available to others, upon written request, from R.D.N.

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Neural biomarkers for assessing different types of imagery in pictorial health warning labels for cigarette packaging: A cross-sectional study.

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Abstract

Objective Countries around the world have increasingly adopted pictorial health warning labels (HWLs) for tobacco packages to warn consumers about smoking-related risks. Research on how pictorial HWLs work has primarily analyzed self-reported responses to HWLs; studies at the neural level comparing the brain's response to different types of HWLs may provide an important complement to prior studies, especially if self-reported responses are systematically biased. In this study we characterize the brain's response to three types of pictorial HWLs for which prior self-report studies indicated different levels of efficacy.

Methods Current smokers rated pictorial HWLs and then observed the same HLWs during functional magnetic resonance (fMRI) scanning. Fifty 18- to 50-year-old current adult smokers who were free from neurological disorders were recruited from the general population and participated in the study. Demo~~graphic~~graphics, smoking-related behaviors, and self-reported ratings of pictorial HWL stimuli were obtained prior to scanning. Brain responses to HWLs were assessed using fMRI, focusing on *a priori* ~~regions of interest~~ROIs.

Results Pictorial HWL stimuli elicited activation in a broad network of brain areas associated and visual processing and emotion. Participants who rated the stimuli as more emotionally arousing also showed greater neural responses at these sites.

Conclusions Self-reported ratings of pictorial HWLs are correlated with neural responses in brain areas associated with visual and emotional processing. Study results cross-validate self-reported ratings of pictorial HWLs and provide insights into how pictorial HWLs are processed.

Strengths and limitations of this study

- This is the first study to explore the relationship between self-reported ratings of pictorial HWLs and neural responses to pictorial HWLs in a large sample (N = 50) of current adult smokers.

- This paper demonstrates the amygdala is maximally activated by pictorial HWLs that depict human ~~suffering~~suffering, followed by images that depict ~~graphic~~graphic effects of smoking, followed by ~~symbolic~~symbolic images of the negative consequences of smoking.
- This paper demonstrates that neural responses to pictorial HWLs attenuate with repeated exposure in most brain regions, but that this response is different in the amygdala.
- Further research is required in order to determine i) exactly why pictorial HWLs depicting human ~~suffering~~suffering elicited such robust responses in the amygdala and ii) whether differential adaptation to ~~Symbolic~~symbolic stimuli is relevant to the creation of optimal HWLs.

INTRODUCTION

According to the World Health Organization, smoking remains the leading cause of preventable death in the Western world.(1, 2) Smoking increases the risk of many non-communicable diseases both in smokers and in those who breathe second hand smoke.(3)[3] To help prevent tobacco use and its consequences, the World Health Organization Framework Convention on Tobacco Control (WHO FCTC) has recommended including prominent, pictorial health warning labels (HWLs) on tobacco packaging to communicate the adverse effects of smoking to consumers and to discourage smoking.(2)Experimental and observational research indicate that HWLs with pictorial imagery are *more* effective than text-only HWLs in both promoting smoking cessation and preventing the initiation of smoking behavior.(4-7)[4–7] A key advantage of pictorial HWLs is likely due to their ability to elicit stronger emotional responses than text-only HWLs.(8)

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The increasing adoption of pictorial HWLs around the world has created a critical need for research designed to i) evaluate the relative effectiveness of different types of HWL content and ii) explain why some HWL content appears more effective than other content. Such research should guide the selection of HWL content, including the rotation of new HWL content over time. Some experimental research has found the self-reported effectiveness of pictorial HWLs is highest when it contains ~~graphic~~graphic images that depict the physical effects of smoking, followed by imagery of personal ~~suffering~~suffering (usually including a face), and finally by ~~symbolic~~symbolic representations of smoking effects that use abstract imagery or symbols.(9-12)These findings are consistent with some observational studies indicating that ~~graphic~~graphic depictions of smoking consequences work best.(13, 14)

The *primary goal* of the current experiment was to explicitly map neural responses to HWLs that contain three different subtypes of imagery that are frequently used in tobacco control communications, including HWLs on cigarette packaging: ~~graphic~~graphic representation of physical consequences of smoking; personal ~~suffering~~suffering from smoking-related consequences; and ~~symbolic~~symbolic representations of risk. Given the visual and emotional nature of pictorial HWLs, we formulated a set of *a priori* ~~*regions of interest*~~*ROIs* (ROIs) that we expected to respond to participants' observations of HWLs, including the amygdala, insula and visual cortex. Converging evidence from numerous neuroscientific investigations confirms a prominent role for the *amygdala* in emotional processing in a number of sensory modalities.(15-19) The amygdala plays a particularly important role in the processing of visual stimuli related to threat and fear.(20-22) We expected that amygdala responses would be driven by our stimuli to the extent that they elicited arousal, fear and perceived threat (e.g., ~~graphic~~graphic HWL vs. ~~symbolic~~symbolic HWL). We also expected pictorial HWLs to elicit robust activity in the *insula*.

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This area has been linked to the experience of disgust, and strongly responds to pictures of mutilation and contamination.(23-26) Finally, based on a prior investigations of the neural response to emotional pictures, we expected the *visual association cortex* to be robustly activated by the presentation of pictorial HWLs.(27-29) We expected all three subtypes of HWLs to elicit a significant response (relative to rest) in this subset of *a priori* ~~regions of interest~~ROIs.

Our *secondary goal* was to examine the relationship between self-report data indicating that HWLs that use ~~graphic~~graphic imagery are more effective than HWLs depicting human ~~suffering~~suffering, which were in turn more effective than ~~symbolic~~symbolic HWLs. We hypothesized that the neural response in our *a priori* ~~regions of interest~~ROIs would differentiate between our three types of HWL (~~Graphic~~graphic > ~~Suffering~~suffering > ~~Symbolic~~symbolic), and that participants who rated pictorial HWL stimuli as more emotionally arousing exhibit heightened activity in these areas. In order to examine these questions, 50 current adult smokers self-reported emotional arousal of HWLs of each pictorial subtype and subsequently observed the same stimuli while their brain activity was measured using fMRI.

METHOD

Participants

Fifty adult smokers between the ages of 18 and 50 (24 females, Mean Age = 27.57) took part in this study. Participants were recruited from the general public, via fliers posted in public locations around the University of South Carolina (USC) and local newspapers. All participants were neurologically healthy smokers with normal to corrected vision. Following initial phone and online screening to confirm qualification for participation, all subjects reported to the McCausland Center and provided informed consent prior to MRI scanning. Following completion of the study protocol, participants were paid $100 for transportation costs related to

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participation in the study. The experiment was performed according to the guidelines of the Declaration of Helsinki and approved by the IRB at USC.

Pictorial HWL Stimuli

A total of 57 pictorial HWLs were used, with images drawn primarily from, based on, or considered for actual HWLs implemented in different countries (**Supplementary Material**).(6, 30, 31) Nineteen pictorial HWLs were developed for each of three pictorial styles: 1) ~~Graphic~~graphic health effect - vivid depiction of physical effects of smoking on the body; 2) Human ~~suffering~~suffering - depiction of personal experience which shows the face and could include the physical, social or emotional impact of smoking-related harm and; 3) ~~Symbolic~~symbolic – representation of message using abstract imagery or symbol. HWL textual content involved short, factual statements based on HWLs that have been implemented and used in prior research.(9)Textual accompaniments addressed 13 different health topics were addressed (i.e., addiction, death, emphysema, gangrene, heart disease, lung cancer, mouth cancer, pregnancy, breast cancer, second hand smoke, strokes, throat cancer, and blindness), with some topics repeated twice within categories (emphysema, death, heart disease, lung cancer, mouth cancer, stroke) Topics and text were counterbalanced across the three pictorial styles. Importantly, the mean luminance values for pictorial HWL s did not differ between subtypes (all p's > 0.18), nor did the overall color (as measured by Red, Green or Blue color values) (all p's > 0.11).

Study Procedures

Demo~~graphic~~graphic Data

All participants were asked a series of standard questions regarding their age, gender, income, ethnicity, and current and past use of cigarettes (**Table 1**).

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Self-reported responses to HWLs

Prior to attending the laboratory session, each participant completed a short survey and rated all 57 HWLs, which were presented online and in random order. The primary reason for collecting the self-report ratings before the fMRI experiment was to minimize respondent burden, as the fMRI protocol lasted an hour. We gauged this as a greater concern than familiarization (which could attenuate subsequent BOLD response), especially as smokers are usually exposed to HWLs many times every day. Negative emotional arousal was assessed by asking participants to rate the HWL on how much it made them afraid ("How much does this warning make you feel afraid?"). As in prior research, (9, 12) participants were also queried concerning ad effectiveness ("How effective is this warning?). For both questions, participants responded with a rating of 1 to 9, with verbal anchors at either end of the rating scale (i.e., 1 = not at all, 9 = extremely).

Smoking Status Screening

To confirm smoking status, carbon monoxide (CO) levels were measured in all participants immediately prior to scanning using a piCO+ Smokerlyzer (Bedfont Scientific, Harrietsham, England). All participants also provided saliva samples immediately prior to scanning to assess cotinine (nicotine metabolite) using liquid chromatography with Tandem Mass Spectrometry (LC-MS/MS). These assays confirmed self-reported smoking status for all participants. Participants also reported the time since last cigarette, the number of days they smoked in the last 30 days, and the average number of cigarettes they smoked per day during that time (**Supplementary Table 1**).

Neural response to HWLs

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During 50 minutes of MRI scanning, each participant completed a single, high resolution structural scan, as well as four functional MRI task runs. Each functional run was 10 minutes and 24 seconds in duration. During the entire scanning run of four runs, each of the 57 images (19 graphic images, 19 suffering images and 19 symbolic images) was presented a total of 10 times each. These images were presented using a block design format. Each block of stimuli was 15 seconds in duration and consisted of the serial presentation of 5 images from the relevant condition (or fixation cross for Rest), separated by 1 second of fixation. A total of 40 blocks (10 graphic images, 10 suffering images, 10 symbolic images and 10 Rest) were presented during each of four functional runs, for a total of 150 HWLs per functional run (50 in each category). The 150 images within a given functional run were randomly chosen from a pool of 600 images created at the beginning of the scanning run. This pool of 600 images consisted of 10 of each individual HWL (10*19*3 = 570), with the remaining 30 being randomly chosen (10 pseudo-random choices from each category-the constraint being that they all had to be different, i.e. no repeats within this subset) (Figure 1) The order of presentation of the blocks within a given functional run was chosen from one of eight pseudo-randomly generated trial orders. These orders were constrained such that i) each condition was equally likely to follow any other condition within a certain functional run; and ii) blocks of the same trial type never occurred more than three times in a row. Each of the four functional runs was identical in duration and content with the exception of the random assignment of images from each condition to its corresponding block. Importantly, the total time (and thus total number of brain volumes recorded) spent showing blocks of each picture type was identical to the total time spent showing Rest blocks.

In order to ensure that participants paid attention to the visual stimuli, we employed a 1-back picture recognition task. Participants were instructed to press a button when the same picture appeared twice in a row. This occurred 5 or 6 times (randomly chosen to prevent participants from assuming they were done detecting repeats within a given run) during each functional run. Placement of repeats was randomized prior to each run using Presentation's built in randomization features.

fMRI Methods

Image Acquisition

All MRI data were collected on a 3T Siemens Trio system with a 12-element head coil. The fMRI (T_2* echo planar imaging) imaging sequence included the following parameters: 320 full brain volumes collected in each of the four 10-minute, 24-second runs; 75° flip angle; time repetition (TR) = 1.95 s; time echo (TE) = 30 ms; in-plane resolution 3.30 × 3.30 mm; slice thickness = 3.0 mm (no gap); 36 axial slices collected in planes aligned parallel to the anterior commissure–posterior commissure line. To improve coregistration of images, all participants were scanned with a high-resolution T_1 MRI, which yielded a 1-mm isotropic image. This sequence had the following parameters: field of view (FOV) = 256 × 256 mm, 192 saggital slices, 9° flip angle, TR = 2250 ms, TE = 4.15 ms.

Data Preprocessing and Modelling

All fMRI data were preprocessed and analyzed using SPM8 (Wellcome Department of Cognitive Neurology, London). Standard preprocessing procedures included image realignment (4^{th} Degree B-Spline Interpolation), coregistration (Mean EPI aligned with T1 then parameters applied to all EPIs), normalization and spatial smoothing (Gaussian Kernel FWHM 8mm). The onsets and durations of each of the conditions of interest were modeled according to the block

design described in the protocol. For our primary analysis, functional data across the four runs was modeled as a boxcar canonically convolved hemodynamic response function (duration 10 seconds). For results regarding between-run differences (i.e. neural adaptation), condition-specific activation within each functional run was modeled as a separate set of events. For all group analyses reported below, we first generated a series contrast images for each individual participant (first level models) and then entered these into random effects models and/or regression models (using SPM's built in general linear model) in order to allow for meaningful population-level inference. First eigen-variates were extracted from second-level models (for each ROI/condition/run) using the VOI toolbox in SPM 8.(32) For the multiple regression analysis between self-reported ratings and neural responses reported below, means for neural responses were calculated at the HWL level (mean values were calculated for each participant for the neural response in each ROI and for each HWL subtype). The resulting parameter estimates were used as the primary dependent variables in the statistical models reported below (i.e. ANOVA and regression analyses).

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RESULTS

Behavioral Performance

Population Variables

Our Participants in the current study were equally spilt with respect to gender (52% Male, 48% Female) and predominantly white (74%, 24% African American, 2% other). The majority of participants (55%) had at least some post-high school education, and were low-income. At the time of scanning, the group's CO levels were 18.74 ppm and cotinine was measured at 207.48 ng/mm confirming that all participants were active smokers. Furthermore, the average participant

smoked 18.74 cigarettes per day, and reported having smoked on 28.32 out of the previous 30 days.

Self-reported Ratings of HWLs

Differences in self-reported emotional arousal across the three stimulus types (~~Graphic~~graphic, ~~Suffering~~suffering, ~~Symbolic~~symbolic) was assessed using one-way within subjects ANOVA, $F(1.44,70.53) = 121.01$, $p < 0.001$. A one-way within subjects ANOVA using perceived effectiveness as a dependent variable and stimulus-type (graphic, suffering, symbolic) as the dependent variable was also significant, $F(1.54,75.27) = 133.27$, $p < 0.001$. For both ANOVAS, post-hoc pair-wise comparisons revealed significant differences between ratings of graphic and suffering stimuli, as well as between ratings of suffering and symbolic stimuli (all p's < 0.01).

Responses to the emotional arousal and perceived effectiveness questions were highly correlated for the ~~Graphic~~graphic ($r(49) = .87$), ~~Suffering~~suffering ($r(49) = .90$) and ~~Symbolic~~symbolic ($r(49) = .90$) stimuli. Because ratings of emotionality were the most relevant for interpretation of our results, we focus on those scores in our analysis section. We would like to note that we did perform the same analyses using perceived effectiveness and obtained a similar pattern of results. (**Figure 2**)

fMRI One-back Task:

One-back task performance data was collected from a total of 176 out of 200 possible fMRI scanning runs (50 participants, with 4 runs per person). Data from 24 of the runs was lost due to experimenter error. We did not exclude the imaging data from these participants as we did monitor the participants' error rates online and ensure they were paying attention (they were just not recorded). A one-way ANOVA using *error rate* as the dependent variable with run as the

factor was not significant, F (3,162) = 1.003, p = 0.393. Moreover, post-hoc comparison of all possible run pairings failed to reveal any significant differences in 1-back performance between n any two runs (all p's > 0.33).

fMRI Response

Primary fMRI Outcomes

Main Effects of HWL Type

In order to isolate cortical networks activated by the presentation of each type of pictorial HWL, we computed a series of contrasts designed to test for the main effects of each of the three stimulus types (~~Graphic~~graphic, ~~Suffering~~suffering, and ~~Symbolic~~symbolic). Specifically, we computed the following contrasts: ~~Graphic~~graphic-Rest, ~~Suffering~~suffering-Rest and ~~Symbolic~~symbolic-Rest (thresholded at p < 0.05 and corrected for family-wise error (FWE)). Observation of pictorial HWL stimuli elicited a significant neural response in a broad network of brain areas including our *a priori* ~~regions of interest~~ROIs (the amygdala, insula, and visual association cortex) as well as a number of other brain areas including the frontal gyrus (inferior, middle, medial, and superior aspects), temporal gyrus (middle and superior), parietal lobe (inferior), supplementary motor area, parahippocampal gyrus, and thalamus. The results of this analysis are listed in **Table 1** and displayed ~~graphic~~graphically in **Figure 3**.

Comparison of HWL-elicited Activation in a priori ROIs

We performed additional analyses in order to identify brain areas whose response properties showed the same pattern as participants' self-reported evaluations of the experimental stimuli in each group (~~Graphic~~graphic > ~~Suffering~~suffering > ~~Symbolic~~symbolic). Accordingly, we performed ROI analyses on our *a priori* ~~regions of interest~~ROIs including the amygdala,

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insula and secondary visual cortex. ~~Regions of interest~~ROIs within the visual association cortex, amygdala and insula were created based on peak activations observed in the contrast comparing the brain's response to all conditions to rest ([~~Graphic~~graphic + ~~Suffering~~suffering + ~~Symbolic~~symbolic] – Rest). All ROIs were centered at the site of peak activation within a given ROI and were spherical in nature (r = 4 mm). A series of one-way within-subjects ANOVAs were used to evaluate neural responses patterns (for ~~Graphic~~graphic, ~~Suffering~~suffering and ~~Symbolic~~symbolic stimuli) within our ROIs. These ANOVAs were significant in the left amygdala, $F(2,98) = 14.59$, $p < 0.001$, right amygdala, $F(2,98) = 21.60$, $p < 0.001$, left insula, $F(2,98) = 4.42$, $p < 0.05$, and visual association cortex, $F(2,98) = 22.69$, $p < 0.001$. As with the behavioral data, we conducted post-hoc pairwise comparisons (all significant results were $p < 0.05$, Bonferroni corrected). In the left amygdala we observed a significant difference between responses in the ~~Graphic~~graphic and ~~Symbolic~~symbolic conditions, as well as in the ~~Suffering~~suffering and ~~Symbolic~~symbolic conditions. In the right amygdala all pair-wise comparisons were significant. In the left amygdala and the visual association cortex, responses to ~~Graphic~~graphic and ~~Symbolic~~symbolic stimuli were significantly different, as were responses to ~~Graphic~~graphic and ~~Suffering~~suffering stimuli. The results of these analyses are shown ~~graphic~~graphically in **Figure 4, A.** We also conducted whole-brain analyses for the following direct comparisons between conditions: graphic > symbolic : symbolic > graphic (**Supplementary Table 2**), suffering > symbolic : symbolic < suffering, (**Supplementary Table 3**) and suffering > graphic : graphic > suffering (**Supplementary Table 4**).

Secondary fMRI Outcomes

Correlation Between Self-Reported Ratings and Neural Response

We ran a series of targeted correlations to determine whether there was a relationship between individual ratings of pictorial HWLs of specific subtypes and the BOLD signal elicited by their presentation. For the ~~graphic~~graphic stimuli, we conducted an SPM multiple regression analysis using individual contrast images for the ~~Graphic~~graphic-Rest condition as the dependent variable and mean self-reported arousal ratings for the ~~Graphic~~graphic HWLs as the independent variable (thresholded at $p < 0.001$, 5 voxel extent). Similar regression analyses were conducted to examine the correlation between HWL ratings and BOLD signal in the ~~Suffering~~suffering and ~~Symbolic~~symbolic conditions. In all three analyses, activation in the right visual association cortex (XYZ_{mni} = -18, -92, 20, XYZ_{mni} = -20, -88, 12, and XYZ_{mni} = -14, -92,12 respectively) was positively correlated with mean ratings of the pictorial HWLs (all r(49)'s > .48) (**Figure 5**). For ~~graphic~~graphic and ~~suffering~~suffering HWLs additional positive correlations were found at sites in the right precentral gyrus (XYZ_{mni} = 44,4,40), r(49) = .45 and r(49) = .42 respectively. For ~~symbolic~~symbolic HWLs there was an additional positive correlation between HWL ratings and activation in the left inferior frontal gyrus (XYZ_{mni} = -52,16,30), r(49) = .37).

Exploratory Analysis of BOLD Signal Adaptation

In addition to examining the main effects of stimulus type, we also conducted a series of 3 (Stimulus) x 4 (Run) repeated measures ANOVAs (one for each ROI) in order to explore possible BOLD signal adaptation to our three stimuli types across the four fMRI runs. The main effect of run was significant for the left insula, $F(3,138) = 11.40$, $p < 0.001$, right insula $F(3,138) = 3.19$, $p < 0.05$, and visual association cortex, $F(3,138) = 15.43$, $p < 0.001$, and nearly significant in the left amygdala, $F(3,138) = 2.66$, $p = 0.074$. There was a significant interaction between Stimulus and Run in both the left amygdala, $F(6,276) = 2.28$, $p < 0.05$, and right

amygdala, $F(6,276) = 2.15$, $p < 0.05$. These results are shown split by run (in order to visualize adaptation) in **Figure 4, B**.

DISCUSSION

Self-reported Ratings of Pictorial HWLs

Results from the current study were generally consistent with prior research using self-reported responses to HWL stimuli. This research consistently indicates that smokers report stronger responses to HWLs with graphic imagery than to symbolic imagery. (10, 11, 31, 33, 34, 37) Results suggesting the greater impact of imagery of suffering than graphic imagery are not necessarily inconsistent with this research. Indeed, a number of the suffering images included graphic elements, and HWLs that combine the two may be may be most effective. (31) Nevertheless, as for self-report research, future fMRI research is needed to determine whether neural responses predict meaning behavioral change (i.e., quitting smoking) or perceptual change (e.g., better understanding of risks, particularly among youth). In general, however, this study suggests that fMRI and self-report produce similar results. One possible concern with the present results is that we did not confirm our specific sample of participants considered each pictorial HWL to belong to one category or another. Future research may consider asking participants to sort pictorial HWLs into categories to address this concern.

Main Effects of HWL Type

The primary goal of the current experiment was to assess neural responses to the presentation of different types of pictorial HWLs that governments have considered for implementation. In general, observation of pictorial HWLs activated large-scale neural networks including the hippocampus, fusiform gyrus, precentral gyrus, supplementary motor area, pars Triangularis, pars opercularis, pars orbitalis and fusiform gyrus. Based on prior literature

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mapping the brain's response to vivid graphic images, we expected all three types of HWLs to elicit activation in the amygdala, the insula and the visual association cortex. Our results are consistent with this literature in that all subtypes of pictorial HWLs used in the current study elicited activation at sites in all three of these areas.

The present study explicitly measured neural responses to observation of pictorial HWLs in a population of confirmed cigarette smokers. Results indicated that pictorial HWLs of all types elicited activation in areas associated with visual processing, as well as the processing of fear and disgust. Activation at sites in the inferior frontal gyrus/precentral gyrus, visual cortex, and to a lesser extent the insula, showed a pattern for strength of response by pictorial stimulus type (i.e., ~~Graphic~~graphic > ~~Suffering~~suffering > ~~Symbolic~~symbolic) that was the same as was found for participants' self-reported ratings of the fear elicited by the stimuli. However, amygdala responses appeared more complex, and it responded maximally to pictorial HWLs depicting human ~~suffering~~suffering, perhaps due to its involvement in empathetic responses (see below). Previous experimental research has found that HWL imagery that combines human ~~suffering~~suffering with ~~graphic~~graphic imagery is rated as more effective than either imagery type alone.(9) In many cases the ~~suffering~~suffering imagery used in our study included ~~graphic~~graphic elements, and that combination may most effectively promote amygdala response. Finally, for all pictorial HWLs, participants that perceived the pictorial HWLs as particularly effective showed heightened activation in the visual association cortex.

Main Effects of HWL Type

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~~The primary goal of the current experiment was to measure the neural response to presentation of pictorial HWLs. Based on prior literature mapping the brain's response to vivid graphic images, we expected the more graphic HWLs to elicit activation in the amygdala, and insula. Our results are consistent with this literature in that all subtypes of pictorial HWLs used in the current study elicited activation at sites in the amygdala, the insula and the visual association cortex.~~

Comparison of HWL-elicited Activation in *a priori* ROIs

Visual Association Cortex

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~~***Region of Interest Analysis***~~

~~A secondary goal of this experiment was to examine the relationship between self-reported ratings of pictorial HWLs with brain data.~~ We expected that responses in regions associated with visual and emotional processing would mirror self-reported ratings of the stimuli (i.e. ~~Graphic~~**graphic** > ~~Sufferings~~**suffering** > ~~Symbolics~~**symbolic**). Results from our ROI analysis were partially consistent with this prediction. Activity in the right visual association cortex did

scale in the same manner as self-reported ratings. The more vivid/~~graphic~~**graphic** nature of certain subtypes of pictorial HWLs may be responsible for the differences we observed in the visual cortex. Images in the ~~Graphic~~**graphic** condition contained more gory/bloody elements than those in any of the other two conditions~~.~~**;** the images in the suffering condition contained a moderate amount of these elements; and images in the symbolic condition contained the least of these elements. We speculate that these negatively valenced elements, which were particularly arousing, may have increased signal in visual areas via afferent projections from the amygdala. It is well established that the amygdala, a key neural pathway for responses to graphic imagery, projects to both primary and secondary visual cortices.(38) It is particularly unlikely that heightened activation in the visual association cortex was caused by differences in low-level features of the images. Neither luminance nor color values for HWL stimuli were significantly different across the three HWL subtypes. Additionally, in at least one previous experiment examining the impact of arousing visual stimuli on visual cortex activity, differences in eye movements did not account for the observed patterns of activation.(28) Therefore it is unlikely that the effects we report were due to differential eye movements.

Amygdala

While responses in the visual association area and insula were consistent with self-reported ratings, activation patterns observed in amygdala were not. Unexpectedly, the amygdala was most robustly activated by suffering HWLs, followed by graphic HWLs, and finally symbolic HWLs. ~~**and the images in the Suffering condition contained a moderate amount of these elements. It is well established that the amygdala, a key neural pathway for responses to graphic imagery, projects to both primary and secondary visual cortices.(33) It is unlikely that this activation was caused by differences in low-level**~~

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~~features of the images because luminance and color values were not significantly different for the three HWL subtypes. Additionally, in at least one previous experiment examining the impact of arousing visual stimuli on visual cortex activity, differences in eye movements did not account for the observed patterns of activation.(28) Therefore it is unlikely that the effects we report were due to differential eye movements.~~

~~While responses in the visual association area and insula were at minimal consistent with self-reported ratings, activation patterns observed in amygdala were not. Surprisingly, the amygdala was most robustly activated by Suffering HWLs, followed by Graphic HWLs, and finally Symbolic HWLs.~~ **As noted in the introduction, the amygdala has been shown to be responsive to arousing stimuli, and fear-evoking stimuli appear to be particularly potent at activating this brain structure. One possibility, then, is that the HWLs that depict personal ~~suffering~~suffering from smoking-related outcomes are best at eliciting fear. However, this is inconsistent with the self-reported data, which indicated that ~~Graphic~~graphic HWLs elicited maximal fear responses. A more parsimonious explanation for this finding is that the relative hyper-activation observed for HWLs with ~~Suffering~~suffering imagery was due to the presence of human faces in the stimuli (all 19 ~~Suffering~~suffering HWLs contained human faces). Lesion, single-cell and whole brain neuroimaging experiments are consistent with the idea that the amygdala is a key component of the face-perception network.(18, 34-39) The amygdala may even process fearful facial stimuli in the absence of conscious processing.(40, 41)** Hence, the inclusion of faces may be particularly important to maintaining arousal-inducing responses under conditions of repeated exposure, as is typically the case with HWLs. Indeed, recent evidence suggests that sustained responses to repeated presentation of emotional faces may be particularly dependent on

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the amygdala.(47) It is also important to note that some of the suffering images (4 of 19) portrayed visible body damage, and thus suffering imagery was not entirely distinct from graphic imagery used in the current experiment, and research based on self-reported ratings indicated that this combination produces the strongest ratings.(31) To better isolate any differential effects of these two image types and the interaction between them, future studies should use imagery that more clearly falls into one category, the other, or both. Another possible explanation for the increased relative amygdala activation observed in the suffering condition relates to stimulus salience (an index of stimulus salience). Studies have demonstrated a strong link between amygdala activation and stimulus salience.(48, 49)

While these results could have implications for the optimization of HWLs, further experiments are necessary to evaluate the predictive validity of fMRI. Future research should aim to separate out the effects of emotionality, salience and human faces by integrating additional conditions (such as neutral images with and without faces). Based on research demonstrating the that BOLD signal in the amygdala is a predictor of subsequent quitting behavior (50) (as is BOLD signal in the medial prefrontal cortex (51, 52)), future prospective studies should examine the extent to which amygdalar BOLD response to the three types of HWLs discussed in the current paper predict changes in smoking behavior or, among youth, perceptions about smoking-related risks. Little research has been conducted with youth before they start smoking, and the strongest effects of HWLs may be due to enhancing aversion for smoking as opposed to changing the behaviors of addicted smokers.

Secondary fMRI Outcomes

Correlation Between Self-Reported Ratings and Neural Response

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An important goal of the present study was to cross-validate self-reported ratings of pictorial HWLs and brain activity recorded during the observation of the same stimuli. This paper is the first to report such results for smoking HWL stimuli. Regarding correlations between self-reported ratings of HWL stimuli and neural activity in our three *a priori* ROIs, only the visual cortex was significant (with the amygdala being nearly significant at p=0.07). We also report significant correlations between behavioral ratings and two additional areas, the junction of the right precentral and inferior frontal gyrus, and the left inferior frontal gyrus pars opercularis.

Visual Association Cortex~~*Faces may be particularly important under conditions of repeated exposure, as with HWLs, as we may be drawn to faces even after repeated exposure, whereas we may be less drawn to graphic bodily harm. Some of the suffering images (4 of 19) portrayed visible body damage, and so Suffering imagery was not entirely distinct from graphic imagery used. To better isolate any differential effects of these two image types and the interaction between them, future studies should use imagery that more clearly falls into one category, the other, or both. Another possible explanation for the increased relative amygdala activation observed in the Suffering condition relates to stimulus salience. Studies have demonstrated a strong link between amygdala activation and stimulus salience.(42, 43) In the context of the current experiment, it may be that images depicting smoking-related suffering were particularly salient to current smokers. While this could have implications for the optimization of HWLs, further experimentation is necessary to evaluate this hypothesis. Future research should aim to separate out the effects of emotionality, salience and human faces by integrating additional conditions (such as neutral images with and without faces). Based on research demonstrating the that BOLD signal in the amygdala is a predictor of subsequent quitting behavior (44) (as is~~

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~~*BOLD signal in the medial prefrontal cortex (45, 46)), it might be useful to conduct future prospective studies that examine the extent to which amygdalar BOLD response to the three types of HWLs discussed in the current paper predict changes in smoking behavior.*~~

An important goal of the present study was to cross-validate self-reported ratings of pictorial HWLs and brain activity recorded during the observation of the same stimuli. This paper is the first to report such results for cigarette HWL stimuli. Regarding correlations between self-reported ratings of HWL stimuli and neural activity in our three *a priori* ROIs, only the visual cortex was significant (with the amygdala being nearly significant at p=0.07). We also report significant correlations between behavioral ratings and two additional areas, the junction of the right precentral and inferior frontal gyrus, and the left inferior frontal gyrus pars opercularis.

Insula and Amygdala~~*To the extent that HWL effectiveness depends on enduring emotional responses, neural adaptation to repeated exposure is an important issue to consider. Our exploratory, post hoc analysis of region-specific adaptation revealed that, in the majority of our regions of interest, BOLD response decreased as a function of repeated exposure to all HWLs. Interestingly, we observed a significant deviation from this pattern in the left and right amygdala. While activation associated with observation of Graphic and Suffering images consistently decreased across the four sessions, activation patterns associated with observation of Symbolic images were less consistent (**Figure 4, B**). It is tempting to speculate that participants did not adapt (neutrally speaking) to repeated presentation of Symbolic stimuli in the same way they adapted to images in the Suffering and Symbolic categories. The abstract nature of these stimuli may have necessitated additional exposure in order to fully process their meaning, and this may account for the observed findings. These data should be interpreted*~~

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~~*cautiously as repeated exposure to HWLs during three, 10-minute scanning sessions may not accurately mimic repeated exposure to HWLs as it exists in real-life (temporally spread out, situation-specific, craving-state-specific, etc.). Further scrutiny of neural adaptation across repeated sessions or repeated days could isolate differences in neural adaptation. If these neural responses can be linked to changes in smoking behavior, public health could be positively impacted.*~~

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~~Relationship Between Neural Measures and Self-Report Data~~

~~**An important goal of the present study was to cross-validate self-reported ratings of pictorial HWLs and brain activity recorded during the observation of the same stimuli. This paper is the first to report such results for smoking HWL stimuli. In general, our correlational data indicate that participants who rated pictorial HWL stimuli (within each category – as opposed to between categories) as more emotionally-arousing showed higher activation of the visual association cortex when viewing the stimuli. This finding is consistent with previous reports demonstrating that activity in the visual cortex is particularly robust during the presentation of emotionally arousing visual stimuli, perhaps due to reentrant enhancement of V2 activity being driven by motivational processes that heighten input from the amygdala. (27, 29, 47)[27–29]**~~

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~~**We also observed an unexpected correlation between self-report ratings and activity at the junction of the right precentral gyrus and inferior frontal gyrus (pars opercularis). Given the location of the activation in the RH (as opposed to the LH which is traditionally associated with such language functions), it is unlikely that heightened responses reflect increased reliance on language. This site is considered to be part of the human mirror neuron system (MNS) and thought to interact with the amygdala and insula when we**~~

~~establish a link between the actions/emotions/intentions of others and our own actions.(48)[49] These stimuli may have been particularly effective at eliciting the types of interpersonal comparisons and or emotions (i.e. empathy) that individuals typically make when seeing the negative effects of their own behaviors in others.(46, 49-51)[49–52] Another possible explanation for the significant correlation we observed between right IFG activity and self-reported ratings is that more emotionally arousing stimuli required greater emotion regulation on the part of the observer. This is consistent with studies reporting recruitment of the right IFG during tasks that require the inhibition of emotions.(52-54)~~

~~Finally, we observed a significant relationship between activity in the left inferior frontal gyrus (BA 44) and self-report ratings of the symbolic stimuli. This area has traditionally been associated with language processing and is active during both overt (i.e. spoken) and covert (i.e. silent) speech.(55-59)[53–57] It is not surprising that symbolic stimuli would utilize language processes. Stimuli of this subtype were the most abstract and likely evoked covert speech during the interpretation process. These data suggest that the Symbolic HWL stimuli that maximally engage language processes are likely to be rated as more arousing than those that do not. If symbolic stimuli are too abstract/confusing to easily verbalize (covertly), then they may be interpreted as more fear eliciting. The involvement of language areas during HWL processing could be the topic of future experiments that assess verbalization during presentation of HWLs of all types.~~

Surprisingly, we did not observe a significant correlation between BOLD signal in the insula or amygdala and self-reported ratings of arousal. However, the correlation between BOLD signal ~~. While we did not find significant correlations between amygdala activity and self-~~

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~~reported ratings of arousal (as might be expected), the correlation between BOLD signal in~~ the right amygdala and self-reported responses in both ~~Graphic~~**graphic** (r(49) = .21, p = 0.07 one-tailed) and ~~Suffering~~**suffering** (r(49) = .20, p = 0.08 one-tailed) conditions was nearly significant~~, and in the predicted direction~~. This failure to reach statistical significance may be due to a number of reasons. One possibility is that the amygdala's response to the emotional stimuli was blunted by the inclusion of text in the HWLs used in the present study. This interpretation is consistent with a comprehensive meta-regression analysis of imaging studies on amygdala activation, which found that presence of language in the stimulus was associated with reduced amygdala activation (as well as greater left lateralization relative to baseline).(54) While the inclusion of text in graphic warning labels has traditionally been justified in terms of added information content (text adds information otherwise not present), it may also be important to examine possible emotional 'blunting' effects that its inclusion may have. Future brain imaging studies might explore this possibility by simultaneously monitoring brain activity and gaze behavior. A better understanding of the how people process graphical and textual elements of HWLs, and how attention to one or the other affects neural processing, particularly after repeated HWL exposure that simulates naturalistic exposure conditions, may help inform the design of future HWLs. ~~It is useful to consider why this correlation might have failed to reach statistical significance. One possibility for this negative finding is that the amygdala's response to the emotional stimuli was blunted by the inclusion of text in the HWLs used in the present study. This interpretation is consistent with a comprehensive meta-regression analysis of imaging studies reporting amygdala activation which found that presence of language in the stimulus was associated with reduced amygdala activation (as well as greater left lateralization relative to baseline).(60) This finding is particularly interesting in light of trends towards the adoption~~

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~~image based HWLs. While the inclusion of text in graphic warning labels has traditionally been justified in terms of added information content (text adds information otherwise not present), it may also be important to examine possible emotional 'blunting' effects that inclusion of text may have.~~

Junction of Right Precentral Gyrus and Inferior Frontal Gyrus

We also observed an unexpected correlation between self-report ratings and activity at the junction of the right precentral gyrus and inferior frontal gyrus (pars opercularis) for suffering HWLs only. Given the location of the activation in the RH (as opposed to the LH which is traditionally associated with such language functions), it is unlikely that heightened responses reflect increased reliance on language. This site is considered to be part of the human mirror neuron system (MNS) and thought to interact with the amygdala and insula when we establish a link between the actions/emotions/intentions of others and our own actions.(48)[49] **One possible explanation for this finding is that suffering stimuli may have been particularly effective at eliciting the types of interpersonal comparisons and or emotions (i.e. empathy) that individuals typically make when seeing the negative effects of their own behaviors in others.(52, 56-58)** Another possible explanation for the significant correlation we observed between right IFG activity and self-reported ratings is that more emotionally arousing stimuli required greater emotion regulation on the part of the observer. This is consistent with studies reporting recruitment of the right IFG during tasks that require the inhibition of emotions. (52-54)

Inferior Frontal Gyrus, Pars Opercularis

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Finally, we observed a significant relationship between activity in the left inferior frontal gyrus (BA 44) and self-report ratings of the symbolic stimuli. This area has traditionally been associated with language processing and is active during both overt (i.e. spoken) and covert (i.e. silent) speech.(55-59)[53–57] It is not surprising that symbolic stimuli would utilize language processes. Stimuli of this subtype were the most abstract and likely evoked covert speech during the interpretation process. The involvement of language areas during HWL processing could be the topic of future experiments that assess verbalization during presentation of HWLs of all types. While it is reasonable to expect that activation of language areas during HWL processing (an indirect measure of covert verbalization) may be related to subsequent behavioral change, future studies will need to address this possibility.

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~~*Future brain imaging could further*~~ *Exploratory Analysis of BOLD Signal Adaptation*

To the extent that HWL effectiveness depends on enduring emotional responses, neural adaptation to repeated exposure may be an important issue to consider. Our exploratory, post-hoc analysis of region-specific adaptation revealed that, in the majority of our ROIs, BOLD response decreased as a function of repeated exposure to all HWLs. Interestingly, we observed a significant deviation from this pattern in the left and right amygdala. While activation associated with observation of graphic and suffering images was higher overall, it consistently decreased across the four runs, whereas activation patterns associated with observation of symbolic images was lower and less consistent (Figure 4, B). Hence, participants may not have adapted (neurally speaking) to repeated presentation of symbolic stimuli in the same way they adapted to images in the suffering and symbolic categories. The abstract nature of symbolic stimuli may have required additional exposures in order to more fully process their meaning, and this may account for the observed findings. These data should be interpreted cautiously, however, as repeated exposure to

HWLs during three, 10-minute scanning runs is unlikely to accurately mimic repeated exposure to HWLs as in real-life, which is temporally spread out, situation specific, and associated with cravings and branding imagery that weakens HWL effects. Future research should more directly examine the relationship between the strength of brain activity elicited by specific subtypes of pictorial HWLs after repeated exposures to HWLs, including more naturalistic exposures that allow for adaptation and habituation.

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Possible Implications for Public Health Policy

Understanding how the brain responds to HWLs can inform the optimal development of HWLs. For example, studies on smokers' neural responses to different types of anti-smoking ads has found that the strength of neural responses elicited by health messaging predicts subsequent individual-level behavioral change as well as the population-level efficacy of different types of ads responses to ads once they are aired in media campaigns.(52) While the current study does not report on behavioral change, future research should. Furthermore, if predictive validity of these methods is established, they could be used to assess the behavioral effects of other types of HWL content. The cost-effectiveness of fMRI compared to self-report studies should also be assessed, particularly if they provide consistent results, as we have found here. Data regarding neural adaptation caused by repeated exposure to pictorial HWLs is could also be important in terms of informing the creation of HWLs designed for maximum long-lasting impact. Arguably, HWLs will only be effective to the extent that they continue to elicit responses from the consumer. Knowing whether or not consumers differentially adapt to different types of HWL content will allow for choice of HWLs that are most likely to discourage smoking.

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Study Limitations

Understanding how the brain responds to HWLs can inform the optimal development of HWLs. For example, studies on smokers' neural responses to different types of anti-smoking ads has found that the strength of neural responses predicts subsequent individual-level cessation behavior(52) as well as population-level cessation attempts (i.e., volume of calls to quitlines) due to different types of ads once they are aired in media campaigns.(67) While the current study does not report on behavioral change, future research should. Furthermore, if the predictive validity of these methods is established, they could be used to evaluate the efficacy of a range of HWL content and presentation styles. The cost-effectiveness of fMRI compared to self-report studies should also be assessed, particularly if they provide consistent results, as we have found here. Data regarding neural adaptation caused by repeated exposure to pictorial HWLs could also be important in terms of informing the creation of HWLs designed for maximum long-lasting impact. HWLs are likely to be most effective if they elicit consumer responses over time. Indeed, the motivation to process messages changes over time, as does the motivation to quit smoking (68) and HWLs effects may become more potent as these motivations change. Knowing more about the process of adaptation to different types of HWL content, including potential differences in the processes of adaptation across diverse groups, may help with designing HWLs that are most likely to discourage smoking.

~~Summary~~General Conclusion

The present study examined self-reported and neural responses to pictorial HWL stimuli of three different types in a population of current adult smokers. Pictorial HWLs elicited robust responses in a broad network of brain sites including those associated with image interpretation (visual association cortex) and emotion (amygdala and insula). Moreover, activation in visual,

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premotor, inferior frontal and to a lesser extent the insular areas, varied in the same manner as self-reported ratings of the stimuli. We report a robust relationship between self-reported ratings of arousal and neural responses, which is important considering that self-reported data can be subject to bias. Our exploratory, post hoc analysis of BOLD signal attenuation across scanning runs revealed differences in the patterns of neural adaptation for different types of HWLs that may be relevant to the optimization of future HWLs. Gaining a better grasp of the relationship between self-reported ratings of HWLs, neural responses elicited by HWLs, and the effectiveness of HWLs should be an important goal of future research.

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NEURAL RESPONSE TO HEALTH WARNING LABELS

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Table 1.

region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
ALL - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**21.62**
Fusiform Gyrus	R	42	-80	-10	19.48
Calcarine	R	12	-94	0	19.02
Hippocampus	R	20	-30	0	15.8
Hippocampus	L	-22	-30	-2	13.73
IFG Pars Triangularis	L	-52	24	30	9.87
Precentral Gyrus	L	-46	-4	52	9.71
Precentral Gyrus	L	-42	8	32	9.26
SMA	L	-6	8	56	8.99
SMA	R	6	10	52	8.53
IFG Pars Triangularis	R	48	24	26	8.67
IFG Pars Opercularis	R	54	22	32	8.66
Middle Frontal Gyrus	R	50	36	24	8.64
Insula	**L**	**-30**	**28**	**2**	**8.39**
IFG Pars Orbitalis	L	-34	30	-8	8.17
IFG Pars Orbitalis	L	-40	26	-12	7.81
Amygdala	**R**	**20**	**-6**	**-14**	**7.33**
Amygdala	**L**	**-22**	**-4**	**-14**	**6.47**
IFG Pars Orbitalis	R	28	30	-10	6.12
Insula	**R**	**32**	**30**	**2**	**5.57**
Fusiform Gyrus	L	-32	-32	-16	6.02
Parahippocampal Gyrus	L	-14	-28	-16	5.13
graphic - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.86**
Declive	L	-38	-70	-10	19.05
Fusiform Gyrus	R	42	-80	-10	18.41
Hippocampus	L	-22	-30	-2	11.35
Hippocampus	R	22	-30	0	13.19
Precentral Gyrus	L	-46	-4	48	9.42
Precentral Gyrus	L	-50	6	38	8.68
Precentral Gyrus	L	-42	6	32	8.47
SMA	L	-6	6	58	8.54
SMA	R	6	10	52	7.87
Precentral Gyrus	R	46	8	34	8.36

Middle Frontal Gyrus	R	50	36	24	8.31
IFG Pars Opercularis	R	54	22	30	7.91
Insula	**L**	**-30**	**30**	**-4**	**7.46**
Parahippocampal Gyrus	R	36	-6	-26	6.54
Amygdala	**L**	**-22**	**-2**	**-16**	**6.38**
Amygdala	**R**	**22**	**-4**	**-14**	**6.1**
Parahippocampal Gyrus	L	-30	-34	-16	5.94
IFG Pars Orbitalis	R	28	30	-10	5.69
Middle Temporal Gyrus	L	-54	-46	8	5.42
suffering - Rest:					
Fusiform Gyrus	R	42	-80	-10	19.19
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.1**
Occipital Lobe (Middle)	**L**	**-26**	**-96**	**8**	**18.46**
Hippocampus	R	24	-28	-2	15.59
Hippocampus	L	-22	-28	-4	14.41
Amygdala	**R**	**20**	**-6**	**-14**	**9.36**
IFG Pars Triangularis	R	52	30	26	9.05
IFG Pars Opercularis	R	46	14	32	8.54
IFG Pars Opercularis	R	52	20	34	7.88
Insula	**L**	**-30**	**28**	**0**	**8.65**
Inferior Frontal Gyrus	L	-36	20	-18	5.25
Precentral Gyrus	L	-46	-4	48	8.48
Precentral Gyrus	L	-40	8	32	8.42
IFG Pars Triangularis	L	-44	18	26	7.72
SMA	R	6	10	52	8.14
Amygdala	**L**	**-20**	**-6**	**-14**	**7.71**
Superior Temporal Gyrus	L	-52	-52	10	7.4
Insula	**R**	**30**	**32**	**-8**	**6.31**
Inferior Parietal Lobule	L	-48	-26	52	5.56
Superior Temporal Gyrus	R	48	-40	10	5.4
symbolic - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.56**
Cuneus	**L**	**-18**	**-100**	**6**	**18.61**
Lingual Gyrus	**R**	**12**	**-94**	**0**	**17.98**
Hippocampus	R	22	-28	-2	14.14
Hippocampus	L	-22	-30	-2	11.36
IFG Pars Triangularis	L	-50	22	30	8.92
IFG Pars Opercularis	L	-42	10	30	8.57
Precentral Gyrus	L	-46	-4	48	8.5
SMA	L	-4	8	56	8.77
SMA	R	6	12	52	8.72
IFG Pars Opercularis	R	54	22	32	7.68

Middle Frontal Gyrus	R	50	36	24	7.59
Precentral Gyrus	R	46	12	32	6.76
Insula	**L**	**-30**	**28**	**0**	**7.28**
IFG Pars Orbitalis	L	-36	28	-10	7.2
Inferior Parietal Lobule	L	-46	-38	54	6.19
Inferior Parietal Lobule	L	-48	-28	52	5.32
Insula	**R**	**32**	**30**	**2**	**5.2**

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute
T-value: local maxima thresholded at $p < 0.05$ FWE corrected, extent threshold $k = 10$
a-priori ROIs indicated in **BOLD.**

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Funding

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Competing Interests Statement

The authors and coauthors have no competing interests to declare.

Contributorship Statement

Roger D. Newman-Norlund helped design the experiment, collected MRI data, analyze behavioral and MRI data, conducted statistical analyses and drafted the paper. James F. Thrasher oversaw the experiment, helped design the experiment, and helped draft the paper. Johann Fridriksson helped design the experiment, recruited participants, collected behavioral and MRI data and revised the draft paper. William Brixius helped collect MRI data and revised the draft paper. Brett E. Froeliger, David Hammond and Michael K. Cummings helped design the experiment and draft the paper.

Data Sharing Statement

All data collected in this experiment is located on the hard drive of the corresponding author, R.D.N. Data analysis is ongoing and the data may still be used for additional papers. After all planned papers have been submitted, the data may be made available to others, upon written request, from R.D.N.

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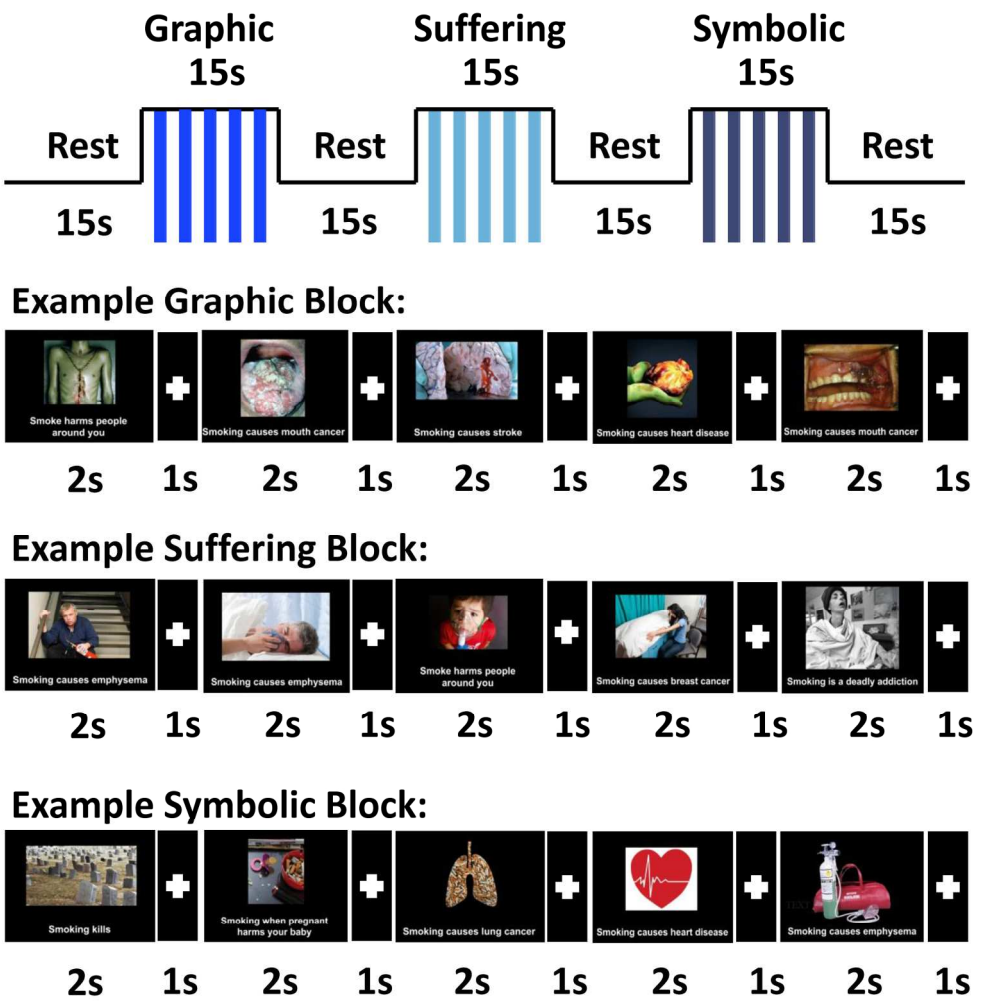

Fig. 1. Graphical representation of the construction of each functional run. All stimuli types (graphic, suffering, and symbolic) were presented in block format. Each block consisted of the presentation of five pseudo-randomly selected stimuli of the appropriate type presented for 2 seconds each, and separated by 1 second of fixation. Block order was pseudo-randomized for each functional run.
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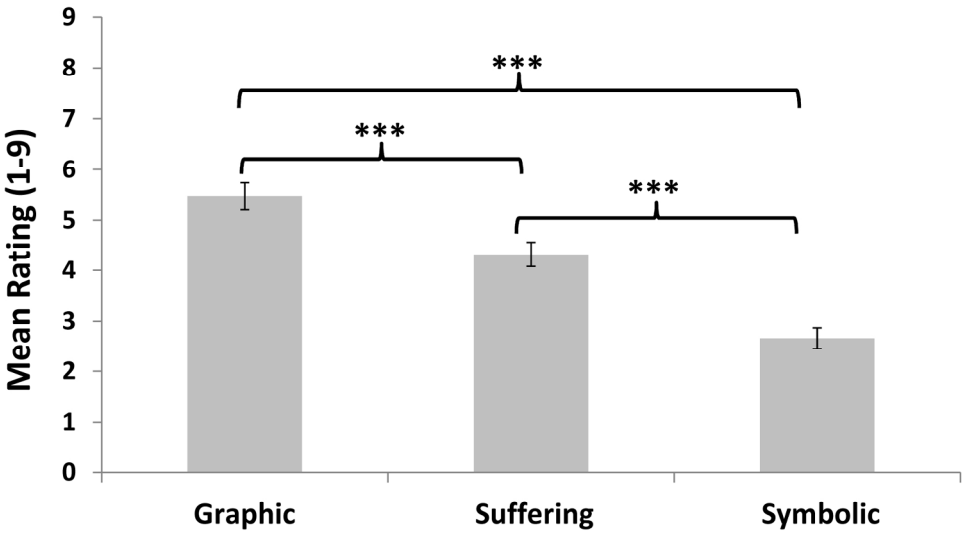


Fig. 2. Behavioral effectiveness ratings of HWLs. All participants rated all HWLs prior to fMRI scanning by responding to the question: "How much does this warning make you feel afraid?". *** = significant p < 0.001 (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM). 229x125mm (227 x 227 DPI)

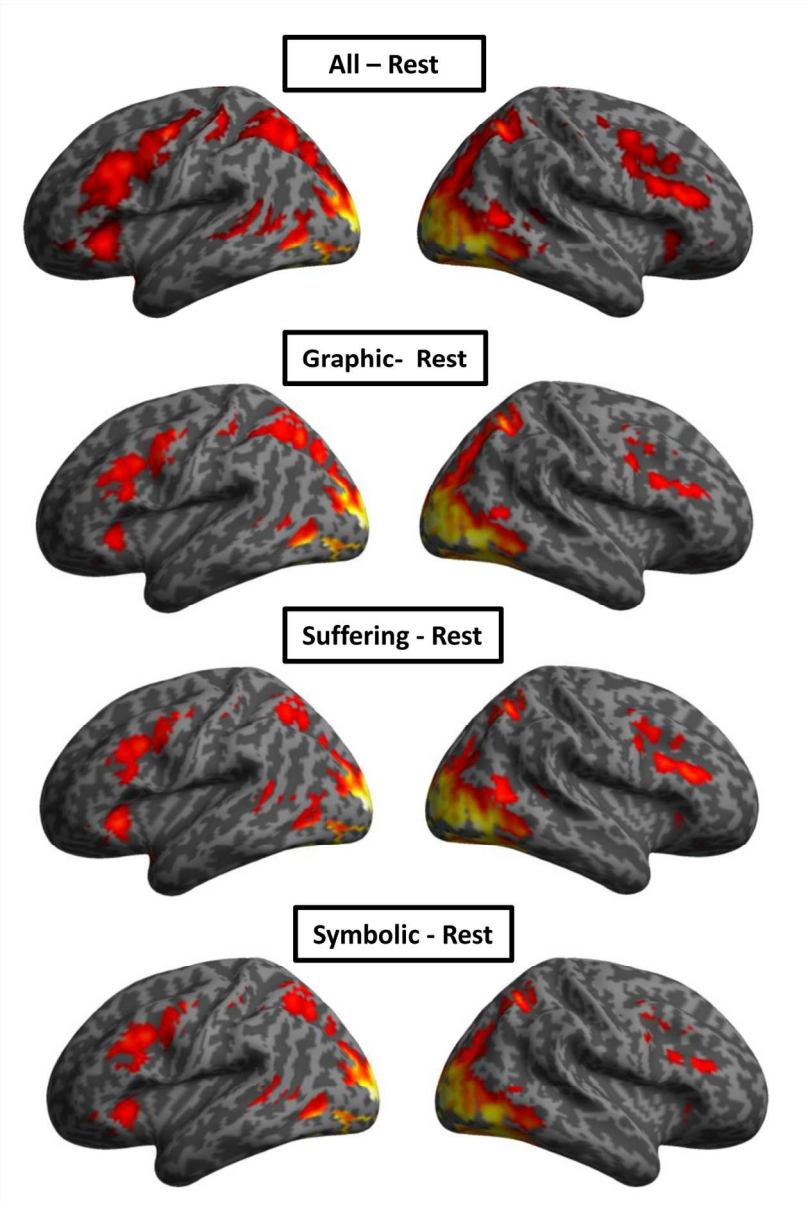


Fig. 3. Main effects of HWLs on BOLD signal (graphic, suffering, symbolic) on BOLD signal. All results are thresholded at p < 0.05 and corrected for family-wise error (FWE). Results are overlaid on a standard inflated brain (cortex_20484.surf.gii) for illustration purposes.
254x377mm (141 x 141 DPI)

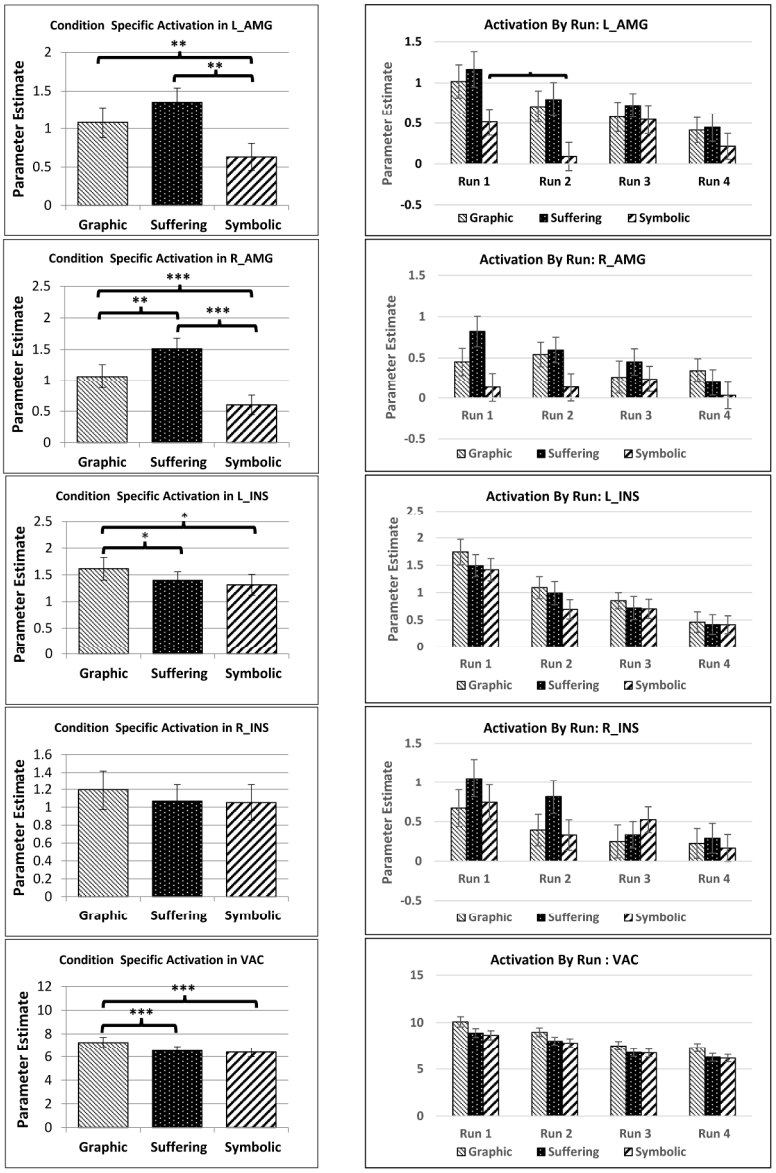


Fig. 4. (A) Results from ROI analyses. (B) Adaptation of BOLD signal in ROIs across four functional scanning runs. L_AMG = left amygdala {XYXmni = -26, -2, -17} , R_AMG = right amygdala {XYXmni = 23, 7, -17}, L_INS = left insula { XYXmni = -30, 30, 4}, R_INS = right insula { XYXmni = 28, 32, -8}, L_OCC = left occipital cortex{XYXmni = -26, -94, 4}, OCC = occipital cortex{XYXmni = -26, -94, 4; XYXmni = 24, -90, -6}, * = significant $p < 0.05$ (within subjects one-tailed t-test), ** = significant $p < 0.05$, *** = significant $p < 0.001$ (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM).
280x421mm (200 x 200 DPI)

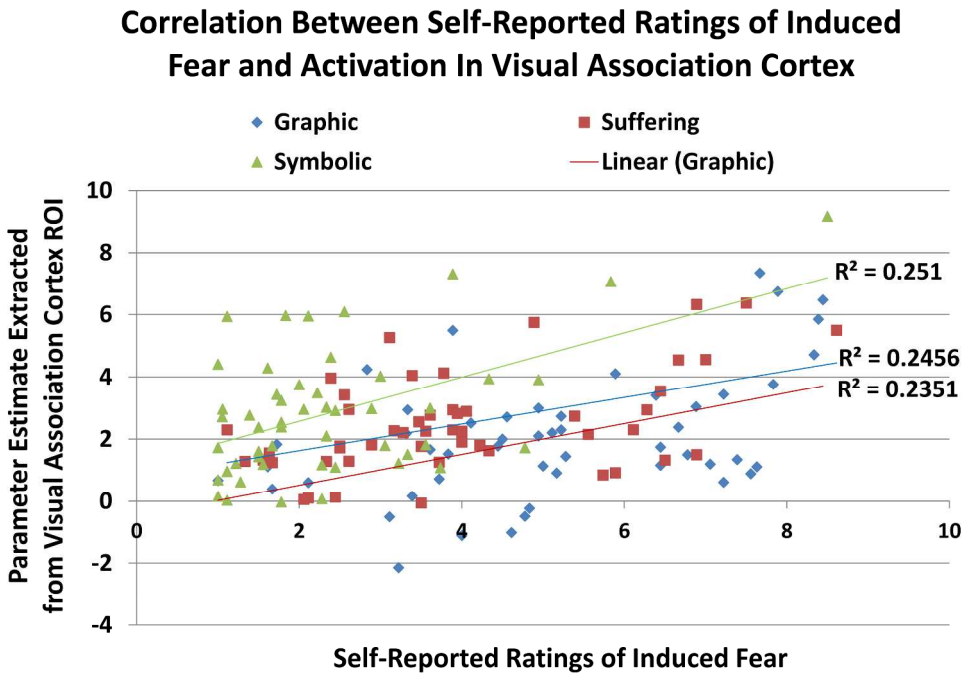


Fig. 5. Correlation between BOLD signal in the visual association cortex (BA 18) and participant self-reported ratings of different subtypes of HWL. The site of maximal correlation between the parameter estimates for the contrast (graphic-Rest) and self-reported ratings of graphic HWL stimuli was located at {XYXmni = -19,-92,20}. The site of maximal correlation between the parameter estimates for the contrast (suffering-Rest) and self-reported ratings of suffering HWL stimuli was located at {XYXmni = -20,-88,12}. The site of maximal correlation between parameter estimates for the contrast (symbolic-Rest) and self-reported ratings of symbolic HWL stimuli was located at {XYXmni = -14,-92,12}.
279x202mm (261 x 261 DPI)

Supplementary Table 1.

Demographic and Smoking Behavior Information		
Demographic Variables		n = 50, mean (SD) or %
sex	% female	48%
age	Mean	27.56
	Range	22
race	% White	74%
	% African American	24%
	% Other	2%
Education	High school or less	26%
	some college/tech school	55%
	college or more	18%
Income	low	63%
	middle	30%
	high	7%
Smoking/Consumer Behavior		
CO Level (ppm)		18.74 (10.57)
Cotinine Level (ng/mm)		207.48 (173.27)
Days Smoked (last 30 days)		28.32 (4.63)
Cigarettes (per day)		14.90 (10.09)
How worried smoking affects health?	not at all	0%
	a little worried	48%
	very worried	52%
Pay attention to HWLs	not at all	54%
	a little worried	40%
	somewhat	4%
	a lot	2%

Supp. Tbl. 1. Demographic and behavior information.

Supplementary Table 2.

Region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Graphic > Symbolic:					
*Lingual Gyrus	L	-16	-90	-8	11.98
*Primary Visual Cortex	R	22	-96	4	10.66
*Superior Parietal Lobule	L	-22	-70	40	6.07
*Superior Parietal Lobule	R	22	62	48	5.6
Inferior Parietal Lobule	L	-34	-38	44	4.69
Supramarginal Gyrus	R	60	-18	40	4.51
Amygdala	R	22	-4	-14	4.15
Precentral Gyrus	R	44	8	28	4.03
Inferior Parietal Lobule	L	-52	-28	36	3.96
Postcentral Gyrus	R	46	-30	44	3.76
Precentral Gyrus	L	-44	4	30	3.64
Amygdala	L	-20	-4	-12	3.6
Symbolic > Graphic:					
*Cuneus	R	4	-82	30	8.36
*Lingual Gyrus	R	10	-66	2	7.14
*Calcarine Gyrus	L	-8	-72	10	6.23
Supramarginal Gyrus	L	50	-34	22	4.63
Anterior Cingulate Gyrus	R	10	34	4	4.42
Middle Temporal Gyrus	R	54	-22	-6	4.40
Superior Temporal Gyrus	L	-52	-4	-12	4.27
IFG Pars Orbitalis	R	40	48	-4	3.74

L: left hemisphere; **R**: right hemisphere; **MNI :** Montreal Neurological Institute; **IFG** : Inferior frontal gyrus.
T-value: local maxima thresholded at $p < 0.001$, uncorrected, extent threshold $k = 10$
*values were significant after FWE correction, extent thresholding $k = 10$

Supp. Tbl. 2. Table of brain activations elicited by observation when comparing Graphic HWLs to Symbolic HWLs.

Supplementary Table 3.

Region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Suffering > Symbolic:					
*Fusiform Gyrus	R	42	-46	-18	8.99
*Post Middle Temporal Gyrus	R	54	-64	12	8.95
*Amygdala	R	20	-6	-10	7.85
*Precuneus	R	4	-58	38	7.03
*Hippocampus	L	-18	-8	-12	6.92
*Occipital Lobe	L	-46	-70	16	6.7
*IFG Pars Triangularis	R	42	18	24	5.89
*Hippocampus	R	18	-32	0	5.31
Ant. Middle Temporal Gyrus	R	58	0	-16	4.36
Orbital Frontal Gyrus	L	-2	56	-12	4.22
IFG Pars Triangularis	R	50	38	14	4.19
Cuneus	R	14	-95	14	3.96
Symbolic > Suffering:					
*Lingual Gyrus	L	-24	-58	-14	6.97
Lingual Gyrus	R	24	-58	-10	5.12
IFG Pars Triangularis	L	-38	42	10	4.78
Occipital Lobe	L	-30	-88	16	4.77
Anterior Cingulate	R	10	36	14	4.16
Superior Frontal Gyrus	R	22	50	10	3.70

L: left hemisphere; **R**: right hemisphere; **MNI :** Montreal Neurological Institute: **Ant.** : Anterior; **Post.** : Posterior; **IFG** : Inferior frontal gyrus.
T-value: local maxima thresholded at $p < 0.001$, uncorrected, extent threshold $k = 10$
*values were significant after FWE correction, extent thresholding $k = 10$

Supp. Tbl. 3. Table of brain activations elicited by observation when comparing Suffering HWLs to Symbolic HWLs.

Supplementary Table 4.

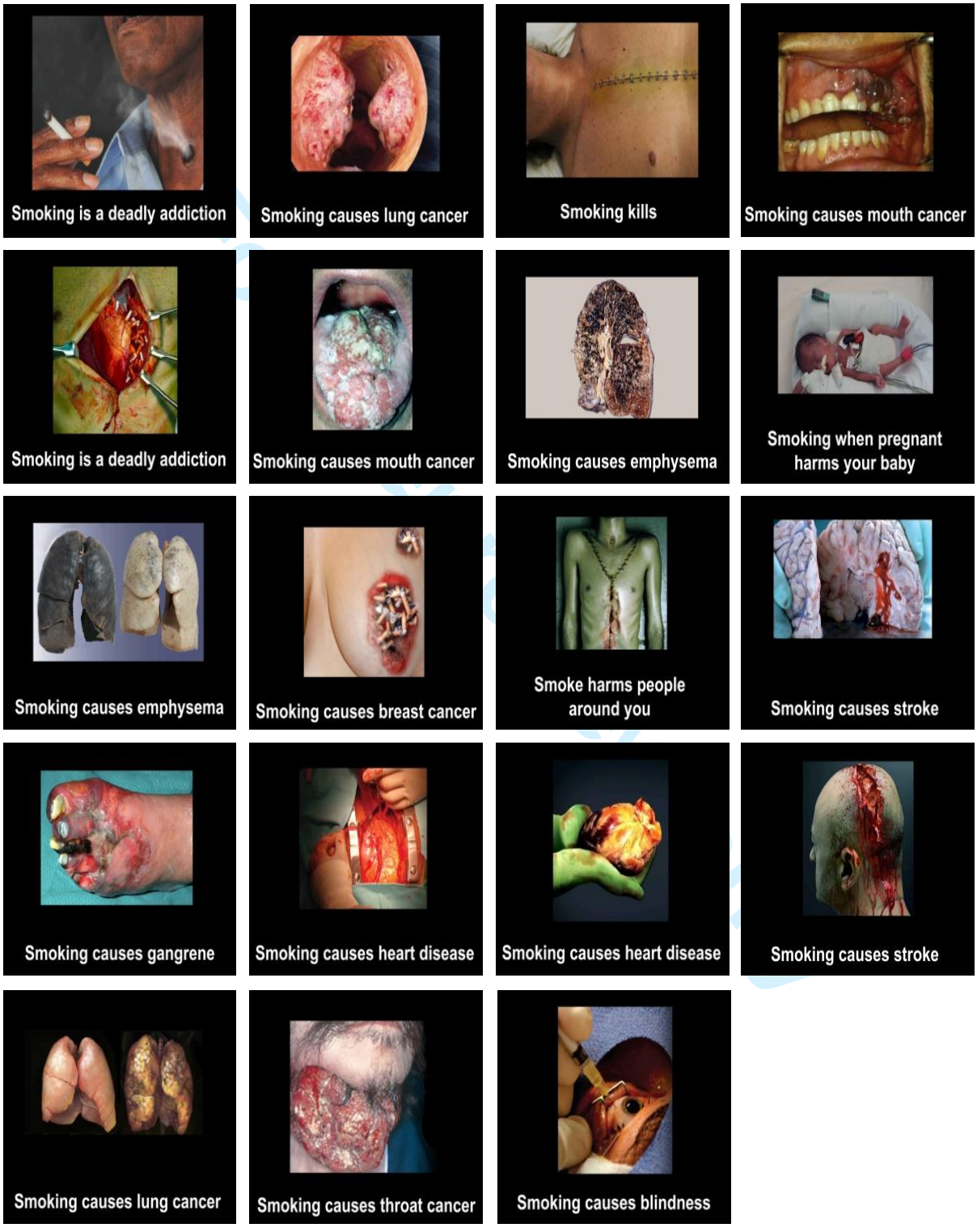
Region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Suffering > Graphic:					
*Post Middle Temporal Gyrus	R	50	-46	12	8.55
*Precuneus	R	4	-60	38	7.48
*Ant. Middle Temporal Gyrus	L	-54	-6	-12	6.87
*Ant. Middle Temporal Gyrus	R	56	-2	-16	6.42
*Post Middle Temporal Gyrus	L	-50	-50	12	6.39
*Orbital Frontal Gyrus	R	4	48	-12	6.20
*Lingual Gyrus	L	-12	-52	0	5.88
*Lingual Gyrus	R	12	-54	2	5.87
*Fusiform Gyrus	L	40	-45	-15	5.59
*Ant. Superior Temp. Gyrus	R	38	20	-28	5.43
*IFG Pars Triangularis	R	52	34	6	5.32
*Ant. Superior Temp. Gyrus	L	-46	10	-20	5.13
Hippocampus	R	28	-8	-14	4.77
Hippocampus	L	-20	-10	-14	4.24
Supplementary Motor Area	L	-2	-24	66	3.66
Graphic > Suffering:					
*Occipital Lobe	L	-30	-86	16	11.29
*Occipital Lobe	R	34	-82	12	10.67
*Fusiform Gyrus	L	-26	-56	-14	10.29
*Fusiform Gyrus	R	26	-56	-12	8.11
*Superior Parietal Lobe	R	26	-66	54	7.91
*Superior Parietal Lobe	L	-24	-74	36	7.24
*Inferior Temporal Gyrus	R	50	-56	-8	6.68
IFG Pars Opercularis	L	-46	2	30	5.03
Inferior Parietal Lobe	L	-40	-40	44	4.36
Middle Frontal Gyrus	L	-44	42	14	3.84
Supramarginal Gyrus	R	44	-32	44	3.77
Supramarginal Gyrus	R	50	-24	44	3.61

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute; **Ant.** : Anterior; **Post.** : Posterior; **IFG** : Inferior frontal gyrus.
T-value: local maxima thresholded at $p < 0.001$, uncorrected, extent threshold $k = 10$
*values were significant after FWE correction, extent thresholding $k = 10$

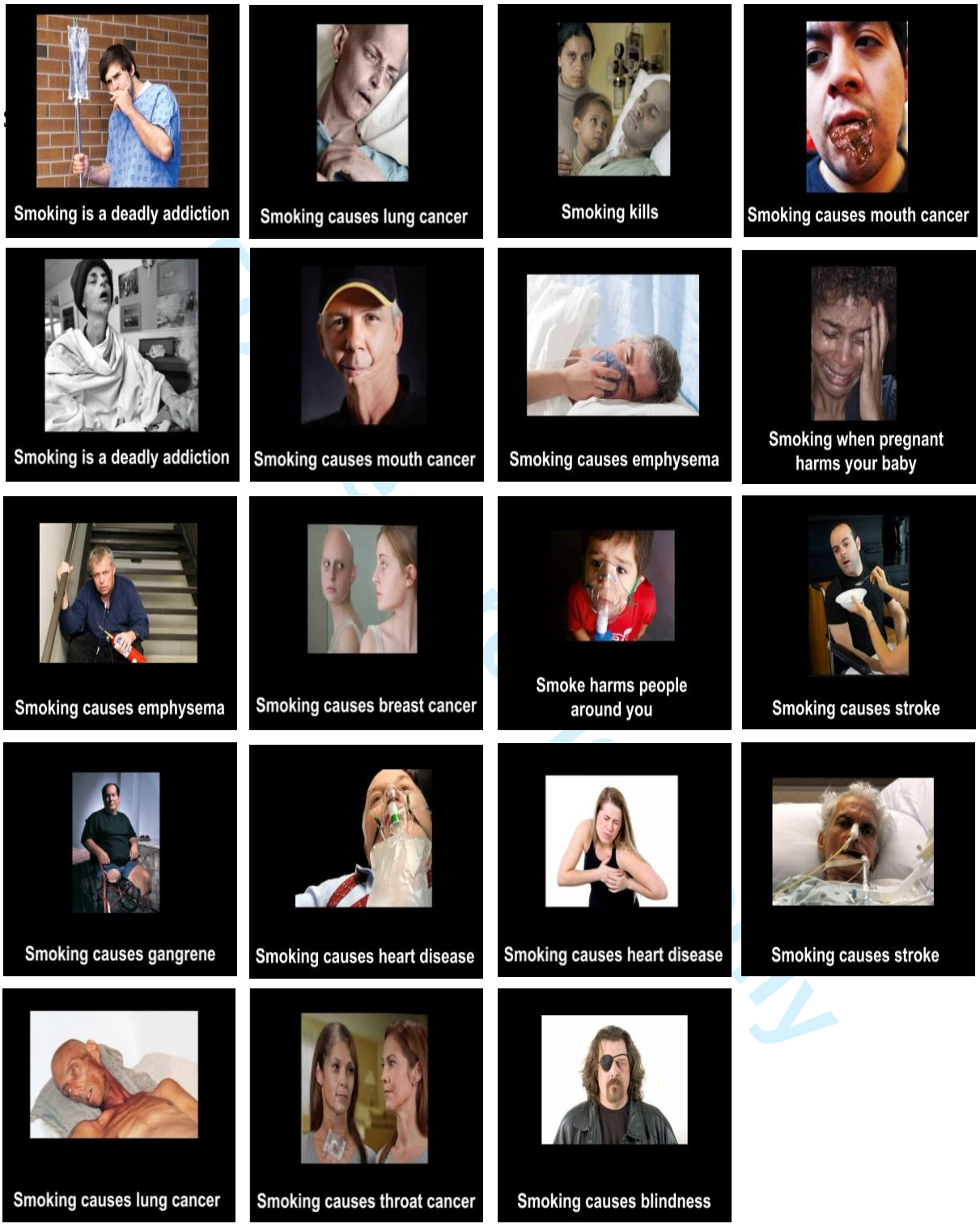
Supp. Tbl. 4. Table of brain activations elicited by observation when comparing Graphic HWLs to Suffering HWLs.

Supplementary Figure 1

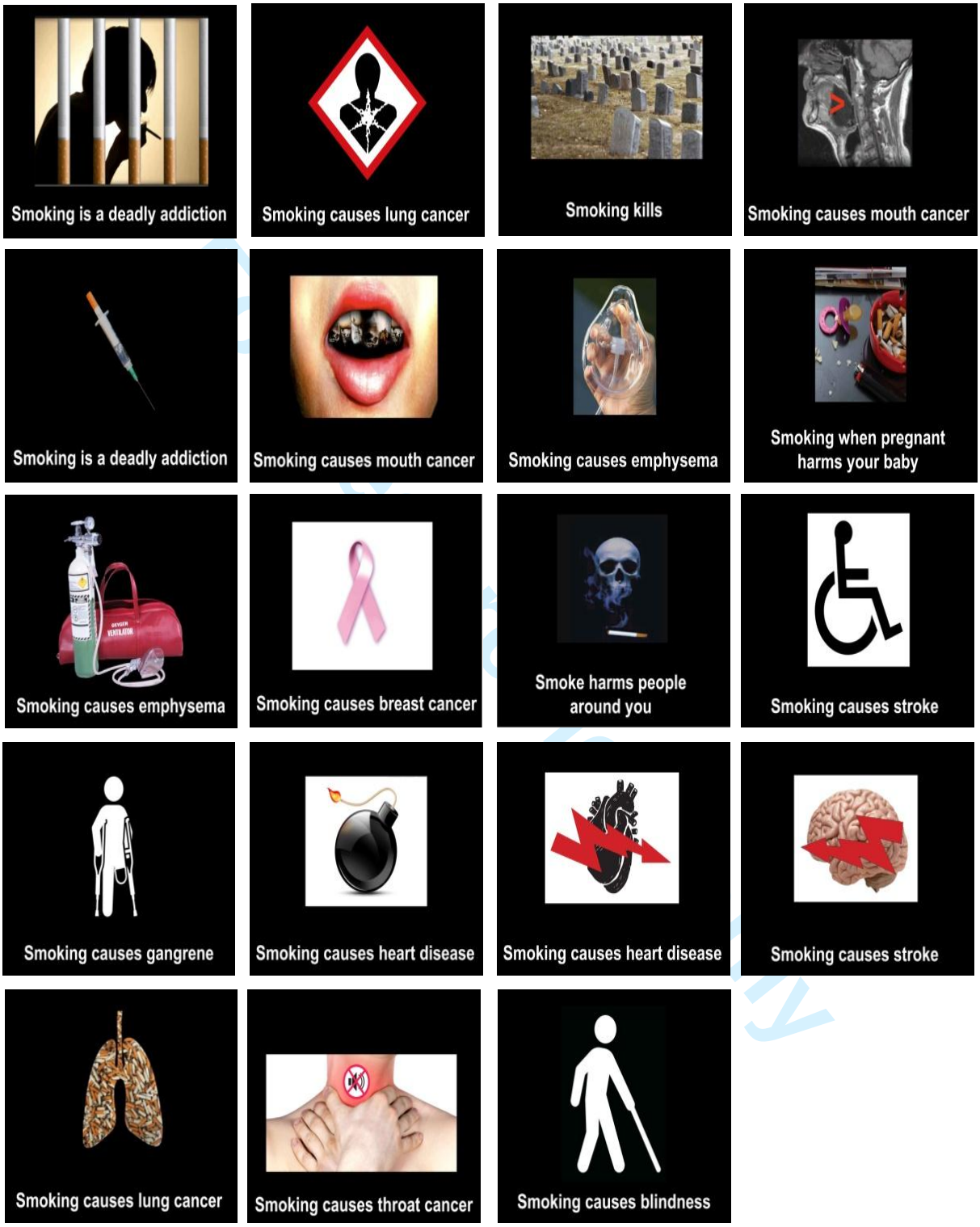
Graphic Images



Suffering Images



Symbolic Images



We confirm our compliance with the following STROBE statement recommendations for reporting cross-sectional studies.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a

		meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Neural biomarkers for assessing different types of imagery in pictorial health warning labels for cigarette packaging: A cross-sectional study.

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Abstract

Objective Countries around the world have increasingly adopted pictorial health warning labels (HWLs) for tobacco packages to warn consumers about smoking-related risks. Research on how pictorial HWLs work has primarily analyzed self-reported responses to HWLs; studies at the neural level comparing the brain's response to different types of HWLs may provide an important complement to prior studies, especially if self-reported responses are systematically biased. In this study we characterize the brain's response to three types of pictorial HWLs for which prior self-report studies indicated different levels of efficacy.

Methods Current smokers rated pictorial HWLs and then observed the same HLWs during functional magnetic resonance (fMRI) scanning. Fifty 18- to 50-year-old current adult smokers who were free from neurological disorders were recruited from the general population and participated in the study. Demographics, smoking-related behaviors, and self-reported ratings of pictorial HWL stimuli were obtained prior to scanning. Brain responses to HWLs were assessed using fMRI, focusing on *a priori* regions of interest.

Results Pictorial HWL stimuli elicited activation in a broad network of brain areas associated and visual processing and emotion. Participants who rated the stimuli as more emotionally arousing also showed greater neural responses at these sites.

Conclusions Self-reported ratings of pictorial HWLs are correlated with neural responses in brain areas associated with visual and emotional processing. Study results cross-validate self-reported ratings of pictorial HWLs and provide insights into how pictorial HWLs are processed.

Strengths and limitations of this study

- This is the first study to explore the relationship between self-reported ratings of pictorial HWLs and neural responses to pictorial HWLs in a large sample (N = 50) of current adult smokers.
- This paper demonstrates the amygdala is maximally activated by pictorial HWLs that depict human suffering, followed by images that depict graphic effects of smoking, followed by symbolic images of the negative consequences of smoking.
- This paper demonstrates that neural responses to pictorial HWLs attenuate with repeated exposure in most brain regions, but that this response is different in the amygdala.
- Further research is required in order to determine i) exactly why pictorial HWLs depicting human suffering elicited such robust responses in the amygdala and ii) whether differential adaptation to symbolic stimuli is relevant to the creation of optimal HWLs.

INTRODUCTION

According to the World Health Organization, smoking remains the leading cause of preventable death in the Western world.(1, 2) Smoking increases the risk of many non-communicable diseases both in smokers and in those who breathe second hand smoke.(3) To help prevent tobacco use and its consequences, the World Health Organization Framework Convention on Tobacco Control (WHO FCTC) has recommended inclusion of prominent, pictorial health warning labels (HWLs) on tobacco packaging to communicate the adverse effects of smoking to consumers and to discourage smoking.(2) Experimental and observational research indicate that HWLs with pictorial imagery are *more* effective than text-only HWLs in both promoting smoking cessation and preventing the initiation of smoking behavior.(4-7) A key advantage of pictorial HWLs is their ability to elicit stronger emotional responses than text-only HWLs.(8)

The increasing adoption of pictorial HWLs around the world has created a critical need for research designed to: i) evaluate the relative effectiveness of different types of HWL content; and ii) explain why some HWL content appears to be more effective than other content. Such research should guide the selection of HWL content, including the rotation of new HWL content over time. Some experimental research has found the self-reported effectiveness of pictorial HWLs is highest when it contains graphic images that depict the physical effects of smoking, followed by imagery of personal suffering (usually including a face), and finally by symbolic representations of smoking effects that use abstract imagery or symbols to represent risk.(9-12) These findings are consistent with observational studies indicating that graphic depictions of smoking consequences work best.(13, 14)

The *primary goal* of the current experiment was to explicitly map neural responses to HWLs that contain three different subtypes of imagery that are frequently used in tobacco control communications, including HWLs on cigarette packaging: graphic representation of physical consequences of smoking; personal suffering from smoking-related consequences; and symbolic representations of risk. Given the visual and emotional nature of pictorial HWLs, we formulated a set of *a priori regions of interest* (ROIs) that we expected to respond to participants' observations of HWLs, including the amygdala, insula and visual association cortex. Converging evidence from numerous neuroscientific investigations confirms a prominent role for the *amygdala* in emotional processing in a number of sensory modalities.(15-19) The amygdala plays a particularly important role in the processing of visual stimuli related to threat and fear.(20-22) We expected that amygdala responses would be driven by our stimuli to the extent that they elicited arousal, fear and perceived threat (e.g., graphic HWL vs. symbolic HWL). We also expected pictorial HWLs to elicit robust activity in the *insula*. This area has been linked to the experience of disgust, and strongly responds to pictures of mutilation and contamination.(23-26) Finally, based on a prior investigations of the neural response to emotional pictures, we expected the *visual association cortex* to be robustly activated by the presentation of pictorial HWLs.(27-29) We expected all three subtypes of HWLs to elicit a significant response (relative to rest) in this subset of *a priori* ROIs.

Our *secondary goal* was to examine the relationship between self-report data indicating that HWLs that use graphic imagery are more effective than HWLs depicting human suffering, which are in turn more effective than symbolic HWLs. We hypothesized that the neural response in our *a priori* ROIs would differentiate between our three types of HWL (graphic > suffering > symbolic), and that participants who rated pictorial HWL stimuli as more emotionally arousing

would exhibit heightened activity in these areas. In order to examine these questions, 50 current adult smokers self-reported emotional arousal elicited by HWLs of each pictorial subtype and subsequently observed the same stimuli while their brain activity was measured using fMRI.

METHOD

Participants

Fifty adult smokers between the ages of 18 and 50 (24 females, Mean Age = 27.57) took part in this study. Participants were recruited from the general public, via fliers posted in public locations around the University of South Carolina (USC) and local newspapers. All participants were neurologically healthy smokers with normal or corrected to normal vision. Following initial phone and online screening to confirm qualification for participation, all subjects reported to the McCausland Center for Brain Imaging and provided informed consent prior to MRI scanning. Following completion of the study protocol, participants were paid $100 for transportation costs related to participation in the study. The experiment was performed according to the guidelines of the Declaration of Helsinki and was approved by the IRB at USC.

Pictorial HWL Stimuli

A total of 57 pictorial HWLs were used, with images drawn primarily from, based on, or considered for actual HWLs implemented in different countries (**Supplementary Figure 1**), including prior HWL research that has relied on self-reported responses to HWLs to determine the efficacy of different content.(6, 30, 31) Nineteen pictorial HWLs were developed for each of three pictorial styles that were matched on textual and topical content: 1) Graphic health effect - vivid depiction of physical effects of smoking on the body; 2) Human suffering - depiction of personal experience which shows the face and could include the physical, social or emotional impact of smoking-related harm and; 3) Symbolic – representation of health risks using abstract

imagery or symbols. Prior HWL research indicates that adult smokers and adolescents discriminate between these three general categories of HWL imagery. (10, 32-36) The textual content to accompany pictorial elements involved short, factual statements based on HWLs that countries have implemented or that have been used in prior research.(9) HWL topics addressed 13 different health issues (i.e., addiction, death, emphysema, gangrene, heart disease, lung cancer, mouth cancer, pregnancy, breast cancer, second-hand smoke, strokes, throat cancer, and blindness), with some topics (emphysema, death, heart disease, lung cancer, mouth cancer, stroke) having two sets of three HWLs on the same health topic but with one of each different pictorial style (graphic, suffering, symbolic). Textual elements were matched across all three HWL subtypes. Importantly, the mean luminance values for pictorial HWL s did not differ between subtypes (all p's > 0.18), nor did the overall color (as measured by Red, Green or Blue color values) (all p's > 0.11).

Study Procedures

Demographic Data

All participants were asked standard questions regarding their age, gender, income, ethnicity, and current and past use of cigarettes (**Supplementary Table 1)**.

Self-reported Responses to HWLs

Prior to attending the laboratory session, each participant completed a short survey and rated all 57 HWLs, which were presented online and in random order. The primary reason for collecting the self-report ratings before the fMRI experiment was to minimize respondent burden, as the fMRI protocol lasted an hour. We gauged this as a greater concern than

familiarization (which could attenuate subsequent BOLD response), especially as smokers are usually exposed to HWLs many times every day. Negative emotional arousal was assessed by asking participants to rate the HWL on how much it made them afraid ("How much does this warning make you feel afraid?"). As in prior research, (9, 12) participants were also queried concerning ad effectiveness ("How effective is this warning?). For both questions, participants responded with a rating of 1 to 9, with verbal anchors at either end of the rating scale (i.e., 1 = not at all, 9 = extremely).

Smoking Status Screening

To confirm smoking status, carbon monoxide (CO) levels were measured in all participants immediately prior to scanning using a piCO+ Smokerlyzer (Bedfont Scientific, Harrietsham, England). All participants also provided saliva samples immediately prior to scanning to assess cotinine (nicotine metabolite) using liquid chromatography with Tandem Mass Spectrometry (LC-MS/MS). These assays confirmed self-reported smoking status for all participants. Participants also reported the time since last cigarette, the number of days they smoked in the last 30 days, and the average number of cigarettes they smoked per day during that time (**Supplementary Table 1**).

Neural Response to HWLs

During 50 minutes of MRI scanning, each participant completed a single, high resolution structural scan, as well as four functional MRI task runs. Each functional run was 10 minutes and 24 seconds in duration. HWLs were presented using a block design format. Each block of stimuli was 15 seconds in duration and consisted of the serial presentation of 5 images from the relevant condition (or fixation cross for Rest), separated by 1 second of fixation. A total of 40

blocks (10 graphic images, 10 suffering images, 10 symbolic images and 10 Rest) were presented during each of four functional runs, for a total of 150 HWLs per functional run (50 in each category). The 150 images within a given functional run were randomly chosen from a pool of 600 images created at the beginning of the scanning session. This pool of 600 images consisted of 10 of each individual HWL (10*19*3 = 570), with the remaining 30 being randomly chosen (10 pseudo-random choices from each category-the constraint being that they all had to be different, i.e. no repeats within this subset). (**Figure 1**) The order of presentation of the blocks within a given functional run was chosen from one of eight pseudo-randomly generated trial orders. These orders were constrained such that i) each condition was equally likely to follow any other condition within a certain functional run; and ii) blocks of the same trial type never occurred more than three times in a row. Each of the four functional runs was identical in duration and content with the exception of the random assignment of images from each condition to its corresponding block. Importantly, the total time (and thus total number of brain volumes recorded) spent showing blocks of each picture type was identical to the total time spent showing Rest blocks.

In order to ensure that participants paid attention to the visual stimuli, we employed a 1-back picture recognition task. Participants were instructed to press a button when the same picture appeared twice in a row. Each functional run contained either 5 or 6 repeated pictures which required the participant to press a button. Placement of repeats was randomized prior to each run using Presentation's built in randomization subroutines.

fMRI Methods

Image Acquisition

All MRI data were collected on a 3T Siemens Trio system with a 12-element head coil. The fMRI (T_2* echo planar imaging) imaging sequence included the following parameters: 320 full brain volumes collected in each of the four 10-minute, 24-second runs; 75° flip angle; time repetition (TR) = 1.95 s; time echo (TE) = 30 ms.; in-plane resolution 3.30 × 3.30 mm; slice thickness = 3.0 mm (no gap); 36 axial slices collected in planes aligned parallel to the anterior commissure–posterior commissure line. To improve coregistration of images, all participants were scanned with a high-resolution T_1 MRI, which yielded a 1-mm isotropic image. This sequence had the following parameters: field of view (FOV) = 256 × 256 mm, 192 saggital slices, 9° flip angle, TR = 2250 ms., TE = 4.15 ms.

Data preprocessing and Modelling

All fMRI data were preprocessed and analyzed using SPM8 (Wellcome Department of Cognitive Neurology, London). Standard preprocessing procedures included image realignment (4^{th} Degree B-Spline Interpolation), coregistration (Mean EPI aligned with T1 then parameters applied to all EPIs), normalization and spatial smoothing (Gaussian Kernel FWHM 8mm). The onsets and durations of each of the conditions of interest were modeled according to the block design described in the protocol. For our primary analysis, functional data across the four runs was modeled as a boxcar canonically convolved hemodynamic response function (duration 10 seconds). For results regarding between-run differences (i.e. neural adaptation), condition-specific activation within each functional run was modeled as a separate set of events. For all group analyses reported below, we first generated a series contrast images for each individual participant (first-level models) and then entered these into random-effects models and/or regression models (using SPM's built in general linear model) in order to allow for meaningful population-level inference. First eigen-variates were extracted from second-level models (for

each ROI/condition/run) using the VOI toolbox in SPM 8.(37) For the multiple regression analysis between self-reported ratings and neural responses reported below, means for neural responses were calculated at the HWL level (mean values were calculated for each participant for the neural response in each ROI and for each HWL subtype). The resulting parameter estimates were used as the primary dependent variables in the statistical models reported below (i.e. ANOVA and regression analyses).

RESULTS

Behavioral Performance

Population Variables

Participants in the current study were equally spilt with respect to gender (52% Male, 48% Female) and predominantly white (74%, 24% African American, 2% other). The majority of participants (55%) had at least some post-high school education, and were low-income. At the time of scanning, the group's CO levels were 18.74 ppm and cotinine was measured at 207.48 ng/mm confirming that all participants were active smokers. Furthermore, the average participant smoked 18.74 cigarettes per day, and reported having smoked on 28.32 out of the previous 30 days.

Self-reported Ratings of HWLs

Differences in self-reported emotional arousal across the three stimulus types (graphic, suffering, symbolic) was assessed using one-way within subjects ANOVA, $F(1.44,70.53) = 121.01$, $p < 0.001$. A one-way within subjects ANOVA using perceived effectiveness as a dependent variable and stimulus-type (graphic, suffering, symbolic) as the independent variable was also significant, $F(1.54,75.27) = 133.27$, $p < 0.001$. For both ANOVAS, post-hoc pair-wise

comparisons revealed significant differences between ratings of graphic and suffering stimuli, as well as between ratings of suffering and symbolic stimuli (all p's < 0.01).

Responses to the emotional arousal and perceived effectiveness questions were highly correlated for the graphic (r(49) = .87), suffering (r(49) = .90) and symbolic (r(49) = .90) stimuli. Because ratings of emotionality were the most relevant for interpretation of our results, we focus on those scores in our analysis section. When the same analyses were conducted using perceived effectiveness, we obtained a similar pattern of results (i.e., graphic > suffering > symbolic). (**Figure 2**)

fMRI One-back Task:

One-back task performance data was collected from a total of 176 out of 200 possible fMRI scanning runs (50 participants, with 4 runs per person). Data from 24 of the runs was lost due to experimenter error. We did not exclude the imaging data from these participants as we did monitor the participants' error rates online and ensure they were paying attention (they were just not recorded). A one-way ANOVA using *error rate* as the dependent variable and run as the factor was not significant, F (3,162) = 1.003, p = 0.393. Moreover, post-hoc comparison failed to reveal any significant differences between error rates in any two runs (all p-values > 0.33).

fMRI Response

Primary fMRI Outcomes

Main Effects of HWL Type

In order to isolate cortical networks activated by the presentation of each type of pictorial HWL, we computed a series of contrasts designed to test for the main effects of each of the three stimulus types (graphic, suffering, and symbolic). Specifically, we computed the following contrasts: graphic-Rest, suffering-Rest and symbolic-Rest (thresholded at p < 0.05 and corrected

for family-wise error [FEW]). Observation of pictorial HWL stimuli elicited a significant neural response in a broad network of brain areas including our *a priori* ROIs (the amygdala, insula, and visual association cortex) as well as a number of other brain areas including the frontal gyrus (inferior, middle, medial, and superior aspects), temporal gyrus (middle and superior), parietal lobe (inferior), supplementary motor area, parahippocampal gyrus, and thalamus. The results of this analysis are listed in **Table 1** and displayed graphically in **Figure 3**.

Comparison of HWL-elicited Activation in a priori ROIs

We performed additional analyses in order to identify brain areas that responded maximally to graphic HWLs, less to suffering HWLs and least to symbolic HWLs. Accordingly, we performed ROI analyses on our *a priori* ROIs including the amygdala, insula and visual association cortex. ROIs within these areas were created based on peak activations observed in the contrast comparing the brain's response to all conditions to rest ([graphic + suffering + symbolic] – Rest).(37) All ROIs were centered at the site of peak activation within a given ROI and were spherical in nature (r = 4 mm). A series of one-way within-subjects ANOVAs were used to evaluate neural responses patterns (for graphic, suffering and symbolic stimuli) within our ROIs. These ANOVAs were significant in the left amygdala, $F(2,98) = 14.59$, $p < 0.001$, right amygdala, $F(2,98) = 21.60$, $p < 0.001$, left insula, $F(2,98) = 4.42$, $p < 0.05$, and visual association cortex, $F(2,98) = 22.69$, $p < 0.001$. As with the behavioral data, we conducted post-hoc pairwise comparisons (all significant results were $p < 0.05$, Bonferroni corrected). In the left amygdala we observed a significant difference between responses in the graphic and symbolic conditions, as well as in the suffering and symbolic conditions. In the right amygdala all pair-wise comparisons were significant. In the left amygdala and the visual association cortex, responses to graphic and symbolic stimuli were significantly different, as were responses to

graphic and suffering stimuli. The results of these analyses are shown graphically in **Figure 4, A.** We also conducted whole-brain analyses for the following direct comparisons between conditions: graphic > symbolic : symbolic > graphic (**Supplementary Table 2**), suffering > symbolic : symbolic < suffering, (**Supplementary Table 3**) and suffering > graphic : graphic > suffering (**Supplementary Table 4**).

Secondary fMRI Outcomes

Correlation Between Self-Reported Ratings and Neural Response

We ran a series of targeted correlations to determine whether there was a relationship between individual ratings of pictorial HWLs of specific subtypes and the BOLD signal elicited by their presentation. For the graphic stimuli, we conducted an SPM multiple regression analysis using individual contrast images for the graphic-Rest condition as the dependent variable and mean self-reported arousal ratings for the graphic HWLs as the independent variable (thresholded at $p < 0.001$, 5 voxel extent). Similar regression analyses were conducted to examine the correlation between HWL ratings and BOLD signal in the suffering and symbolic conditions. In all three analyses, activation in the right visual association cortex (XYZ_{mni} = -18, -92, 20, XYZ_{mni} = -20, -88, 12, and XYZ_{mni} = -14, -92,12 respectively) was positively correlated with mean ratings of the pictorial HWLs (all r(49)'s > .48) (**Figure 5**). For graphic and suffering HWLs additional positive correlations were found at sites in the right precentral gyrus (XYZ_{mni} = 44,4,40), r(49) = .45 and r(49) = .42 respectively. For symbolic HWLs there was an additional positive correlation between HWL ratings and activation in the left inferior frontal gyrus (XYZ_{mni} = -52,16,30), r(49) = .37).

Exploratory Analysis of BOLD Signal Adaptation

In addition to examining the main effects of stimulus type, we also conducted a series of 3 (Stimulus) x 4 (Session) repeated measures ANOVAs (one for each ROI) in order to explore possible BOLD signal adaptation to our three stimuli types across the four fMRI runs. The main effect of run was significant for the left insula, $F(3,138) = 11.40$, $p < 0.001$, right insula $F(3,138) = 3.19$, $p < 0.05$, and visual association cortex, $F(3,138) = 15.43$, $p < 0.001$, and nearly significant in the left amygdala, $F(3,138) = 2.66$, $p = 0.07$. There was a significant interaction between Stimulus and Run in both the left amygdala, $F(6,276) = 2.28$, $p < 0.05$, and right amygdala, $F(6,276) = 2.15$, $p < 0.05$. These results are shown split by run (in order to visualize adaptation) in **Figure 4, B**.

DISCUSSION

Self-reported Ratings of Pictorial HWLs

Results from the current study were generally consistent with prior research using self-reported responses to HWL stimuli. This research consistently indicates that smokers report stronger responses to HWLs with graphic imagery than to symbolic imagery. (10, 11, 32, 34, 35, 38) Results suggesting the greater impact of imagery of suffering than graphic imagery are not necessarily inconsistent with this research. Indeed, a number of the suffering images included graphic elements, and HWLs that combine the two may be may be most effective. (32) Nevertheless, as for self-report research, future fMRI research is needed to determine whether neural responses predict meaningful behavioral change (i.e., quitting smoking) or perceptual change (e.g., better understanding of risks, particularly among youth). In general, however, this study suggests that fMRI and self-report produce similar results. One possible concern with the present results is that we did not confirm our specific sample of participants considered each

pictorial HWL to belong to one category or another. Future research may consider asking participants to sort pictorial HWLs into categories to address this concern.

Main Effects of HWL Type

The primary goal of the current experiment was to assess neural responses to the presentation of different types of pictorial HWLs that governments have considered for implementation. In general, observation of pictorial HWLs activated large-scale neural networks including the hippocampus, fusiform gyrus, precentral gyrus, supplementary motor area, pars triangularis, pars opercularis, pars orbitalis and fusiform gyrus. Based on prior literature mapping the brain's response to vivid graphic images, we expected all three types of HWLs to elicit activation in the amygdala, the insula and the visual association cortex. Our results are consistent with this literature in that all subtypes of pictorial HWLs used in the current study elicited activation at sites in all three of these areas.

Comparison of HWL-elicited Activation in *a priori* ROIs

Visual Association Cortex

We expected the intensity of BOLD signal in regions associated with visual and emotional processing to mirror self-reported ratings of the stimuli (i.e. graphic > suffering > symbolic). Results from our ROI analysis were partially consistent with this prediction. Activity in the right visual association cortex did scale in the same manner as self-reported ratings of the HWL stimuli. The more vivid/graphic nature of certain subtypes of pictorial HWLs may be responsible for the differences we observed in the visual cortex. Images in the graphic condition contained more gory/bloody elements than those in any of the other two conditions; the images in the suffering condition contained a moderate amount of these elements; and images in the symbolic condition contained the least of these elements. We speculate that these negatively

valenced elements, which were particularly arousing, may have increased signal in visual areas via afferent projections from the amygdala. It is well established that the amygdala, a key neural pathway for responses to graphic imagery, projects to both primary and secondary visual cortices.(39) It is particularly unlikely that heightened activation in the visual association cortex was caused by differences in low-level features of the images as neither luminance nor color values for HWL stimuli were significantly different across the three HWL subtypes. Additionally, in at least one previous experiment examining the impact of arousing visual stimuli on visual cortex activity, differences in eye movements did not account for the observed patterns of activation.(28) Therefore it is unlikely that the effects we report were due to differential eye movements.

Amygdala

While responses in the visual association area and insula were consistent with self-reported ratings, activation patterns observed in amygdala were not. Unexpectedly, the amygdala was most robustly activated by suffering HWLs, followed by graphic HWLs, and finally symbolic HWLs. As noted in the introduction, the amygdala has been shown to be responsive to arousing stimuli, and fear-evoking stimuli robustly activate this brain structure. One possibility, then, is that the HWLs depicting personal suffering from smoking-related outcomes are effective at eliciting fear in current adult smokers. However, this is inconsistent with the self-reported data, which indicated that graphic HWLs elicited maximal fear responses. A more parsimonious explanation for this finding is that the relatively higher activation observed for HWLs with suffering imagery was due to the presence of human faces in the stimuli (all 19 suffering HWLs contained human faces). Lesion, single-cell and whole brain neuroimaging experiments are consistent with the idea that the amygdala is a key component of the face-perception

network.(18, 40-45) The amygdala may even process fearful facial stimuli in the absence of conscious processing.(46, 47) Hence, the inclusion of faces may be particularly important to maintaining arousal-inducing responses under conditions of repeated exposure, as is typically the case with HWLs. Indeed, recent evidence suggests that sustained responses to repeated presentation of emotional faces may be particularly dependent on the amygdala.(48) It is also important to note that some of the suffering images (4 of 19) portrayed visible body damage, and thus suffering imagery was not entirely distinct from graphic imagery used in the current experiment, and research based on self-reported ratings indicated that this combination produces the strongest ratings.(32) To better isolate any differential effects of these two image types, as well as the interaction between them, future studies should use imagery that more clearly falls into one category, the other, or both. Another possible explanation for the increased relatively higher amygdala activation observed in the suffering condition relates to stimulus salience. Studies have demonstrated a strong link between amygdala activation and this attribute.(49, 50)

While these results could have implications for the optimization of HWLs, further experiments are necessary to evaluate the predictive validity of fMRI. Future research should aim to separate out the effects of emotionality, salience and human faces by integrating additional conditions (such as neutral images with and without faces). Based on research demonstrating that the BOLD signal in the amygdala is a predictor of subsequent quitting behavior (51) (as is BOLD signal in the medial prefrontal cortex (52, 53)), future prospective studies should examine the extent to which amygdalar BOLD response to the three types of HWLs discussed in the current paper predict changes in smoking behavior or, among youth, perceptions of smoking-related risks. Little research has been conducted with youth before they

start smoking, and the strongest effects of HWLs may be due to enhancing aversion for smoking as opposed to changing the behaviors of addicted smokers.

Secondary fMRI Outcomes

Correlation Between Self-Reported Ratings and Neural Response

An important goal of the present study was to cross-validate self-reported ratings of pictorial HWLs and brain activity recorded during the observation of the same stimuli. This paper is the first to report such results for cigarette HWL stimuli. Regarding correlations between self-reported ratings of HWL stimuli and neural activity in our three *a priori* ROIs, only the visual cortex was significant (with the amygdala being nearly significant at p=0.07). We also report significant correlations between behavioral ratings and two additional areas, the junction of the right precentral and inferior frontal gyrus, and the left inferior frontal gyrus pars opercularis.

Visual Association Cortex

Our correlational data indicate that participants who rated pictorial HWL stimuli (within each category – as opposed to between categories) as more emotionally arousing showed higher activation of the visual association cortex when viewing the stimuli. This finding is consistent with previous reports demonstrating that activity in the visual cortex is particularly robust during the presentation of emotionally arousing visual stimuli, perhaps due to reentrant enhancement of V2 activity being driven by motivational processes that heighten input from the amygdala. (27, 29, 54)

Insula and Amygdala

Surprisingly, we did not observe a significant correlation between BOLD signal in the insula or amygdala and self-reported ratings of arousal. However, the correlation between BOLD

signal in the right amygdala and self-reported responses in both graphic (r(49) = .21, p = 0.07 one-tailed) and suffering (r(49) = .20, p = 0.08 one-tailed) conditions was nearly significant. This failure to reach statistical significance may be due to a number of factors. One possibility is that the amygdala's response to the emotional stimuli was blunted by the inclusion of text in the HWLs used in the present study. This interpretation is consistent with a comprehensive meta-regression analysis of imaging studies on amygdala activation, which found that presence of language in the stimulus was associated with reduced amygdala activation (as well as greater left lateralization relative to baseline).(55) While the inclusion of text in graphic warning labels has traditionally been justified in terms of added information content (text adds information otherwise not present), it may also be important to examine possible emotional 'blunting' effects that its inclusion may have. Future brain imaging studies might explore this possibility by simultaneously monitoring brain activity and gaze behavior. A better understanding of the how people process graphical and textual elements of HWLs, and how attention to one or the other affects neural processing, particularly after repeated HWL exposure that simulates naturalistic exposure conditions, may help inform the design of future HWLs.

Junction of Right Precentral Gyrus and Inferior Frontal Gyrus

We also observed an unexpected correlation between self-report ratings and activity at the junction of the right precentral gyrus and inferior frontal gyrus (pars opercularis) for suffering HWLs only. Given the location of the activation in the RH (as opposed to the LH which is traditionally associated with such language functions), it is unlikely that heightened responses reflect increased reliance on language. This site is considered to be part of the human mirror neuron system (MNS) and thought to interact with the amygdala and insula when a link is established between the actions/emotions/intentions of others and our own actions.(56) One

possible explanation for this finding is that suffering stimuli may have been particularly effective at eliciting the types of interpersonal comparisons and or emotions (i.e. empathy) that individuals typically make when seeing the negative effects of their own behaviors in others.(53, 57-59) Another possible explanation for the significant correlation we observed between right IFG activity and self-reported ratings is that more emotionally arousing stimuli required greater emotion regulation on the part of the observer. This is consistent with studies reporting recruitment of the right IFG during tasks that require the inhibition of emotions. (60-62)

Inferior Frontal Gyrus, Pars Opercularis

Finally, we observed a significant relationship between activity in the left inferior frontal gyrus (BA 44) and self-report ratings of the symbolic stimuli. This area has traditionally been associated with language processing and is active during both overt (i.e. spoken) and covert (i.e. silent) speech.(63-67) It is not surprising that symbolic stimuli would utilize language processes. Stimuli of this subtype were the most abstract and likely evoked covert speech during the interpretation process. The involvement of language areas during HWL processing could be the topic of future experiments that assess verbalization during presentation of HWLs of all types. While it is reasonable to expect that activation of language areas during HWL processing (an indirect measure of covert verbalization) may be related to subsequent behavioral change, future studies will need to address this possibility.

Exploratory Analysis of BOLD Signal Adaptation

To the extent that HWL effectiveness depends on enduring emotional responses, neural adaptation to repeated exposure may be an important issue to consider. Our exploratory, post-hoc analysis of region-specific adaptation revealed that, in the majority of our ROIs, BOLD response decreased as a function of repeated exposure to all HWLs. Interestingly, we observed a

significant deviation from this pattern in the left and right amygdala. While activation associated with the observation of graphic and suffering images was higher overall, it consistently decreased across the four runs, whereas activation patterns associated with the observation of symbolic images was, overall, both less robust and less consistent (**Figure 4, B**). Hence, participants may not have adapted (neurally speaking) to repeated presentation of symbolic stimuli in the same way they adapted to images in the suffering and graphic categories. The abstract nature of symbolic stimuli may have required additional exposures in order to more fully process their meaning, and this may account for the observed findings. These data should be interpreted cautiously, however, as repeated exposure to HWLs during three, 10-minute scanning runs is unlikely to accurately mimic repeated exposure to HWLs as in real-life, which is temporally spread out, situation specific, and associated with cravings and branding imagery that weakens HWL effects. Future research should more directly examine the relationship between the strength of brain activity elicited by specific subtypes of pictorial HWLs after repeated exposures to HWLs, including more naturalistic exposures that allow for adaptation and habituation.

Possible Implications for Public Health Policy and Limitations

Understanding how the brain responds to HWLs can inform the optimal development of HWLs. For example, studies on smokers' neural responses to different types of anti-smoking ads have found that the strength of neural responses elicited by health messaging predicts subsequent individual-level behavioral change as well as the population-level efficacy of different types of ads responses to ads once they are aired in media campaigns.(53) While the current study does not report on behavioral change, future research should. Furthermore, if predictive validity of these methods is established, they could be used to assess the behavioral

effects of other types of HWL content. The cost-effectiveness of fMRI compared to self-report studies should also be assessed, particularly if they provide consistent results, as we have found here. Data regarding neural adaptation caused by repeated exposure to pictorial HWLs could also be important in terms of informing the creation of HWLs designed for maximum long-lasting impact. Arguably, HWLs will only be effective to the extent that they continue to elicit responses from the consumer. Indeed, the motivation to process messages changes over time, as does the motivation to quit smoking (69) and HWLs effects may become more potent as these motivations change. Knowing more about the process of adaptation to different types of HWL content, including potential differences in the processes of adaptation across diverse groups, may help with designing HWLs that are most likely to discourage smoking.

General Conclusion

The present study examined adult smokers' self-reported and neural responses to three different types of pictorial HWL stimuli that governments commonly use on cigarette packaging. Pictorial HWLs elicited robust responses in an extensive network of brain sites including those associated with image interpretation (visual association cortex) and emotion (amygdala and insula). Moreover, activation in visual, premotor, inferior frontal and, to a lesser extent, the insular areas, varied in a manner consistent with self-reported ratings of the stimuli. We report a robust relationship between self-reported ratings of arousal and neural responses, which is important considering that self-reported data can be subject to bias. Our exploratory, post hoc analysis of BOLD signal attenuation across scanning runs revealed differences in the patterns of neural adaptation for different types of HWLs that may be relevant to the optimization of future HWLs. Gaining a better grasp of the relationship between self-reported ratings of HWLs, neural

responses elicited by HWLs, and the effectiveness of HWLs should be an important goal of future research.

Figure Legends

Fig. 1. Graphical representation of the construction of each functional run. All stimuli types (graphic, suffering, and symbolic) were presented in block format. Each block consisted of the presentation of five pseudo-randomly selected stimuli of the appropriate type presented for 2 seconds each, and separated by 1 second of fixation. Block order was pseudo-randomized for each functional run.

Fig. 2. Behavioral effectiveness ratings of HWLs. All participants rated all HWLs prior to fMRI scanning by responding to the question: **"**How much does this warning make you feel afraid?". *** = significant $p < 0.001$ (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM).

Fig. 3. Main effects of HWLs on BOLD signal (graphic, suffering, symbolic) on BOLD signal. All results are thresholded at $p < 0.05$ and corrected for family-wise error (FWE). Results are overlaid on a standard inflated brain (cortex_20484.surf.gii) for illustration purposes.

Fig. 4. (A) Results from ROI analyses. **(B)** Adaptation of BOLD signal in ROIs across four functional scanning runs. L_AMG = left amygdala {XYX_{mni} = -26, -2, -17} , R_AMG = right amygdala {XYX_{mni} = 23, 7, -17}, L_INS = left insula { XYX_{mni} = -30, 30, 4}, R_INS = right insula { XYX_{mni} = 28, 32, -8}, L_OCC = left occipital cortex{XYX_{mni} = -26, -94, 4}, OCC = occipital cortex{XYX_{mni} = -26, -94, 4; XYX_{mni} = 24, -90, -6}, * = significant $p < 0.05$ (within subjects one-tailed t-test), ** = significant $p < 0.05$, *** = significant $p < 0.001$ (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM).

Fig. 5. Correlation between BOLD signal in the visual association cortex (BA 18) and participant self-reported ratings of different subtypes of HWL. The site of maximal correlation between the parameter estimates for the contrast (graphic-Rest) and self-reported ratings of graphic HWL stimuli was located at {XYX_{mni} = -19,-92,20}. The site of maximal correlation between the parameter estimates for the contrast (suffering-Rest) and self-reported ratings of suffering HWL stimuli was located at {XYX_{mni} = -20,-88,12}. The site of maximal correlation between parameter estimates for the contrast (symbolic-Rest) and self-reported ratings of symbolic HWL stimuli was located at {XYX_{mni} = -14,-92,12}.

Table 1.

region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
ALL - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**21.62**
Fusiform Gyrus	R	42	-80	-10	19.48
Calcarine	R	12	-94	0	19.02
Hippocampus	R	20	-30	0	15.8
Hippocampus	L	-22	-30	-2	13.73
IFG Pars Triangularis	L	-52	24	30	9.87
Precentral Gyrus	L	-46	-4	52	9.71
Precentral Gyrus	L	-42	8	32	9.26
SMA	L	-6	8	56	8.99
SMA	R	6	10	52	8.53
IFG Pars Triangularis	R	48	24	26	8.67
IFG Pars Opercularis	R	54	22	32	8.66
Middle Frontal Gyrus	R	50	36	24	8.64
Insula	**L**	**-30**	**28**	**2**	**8.39**
IFG Pars Orbitalis	L	-34	30	-8	8.17
IFG Pars Orbitalis	L	-40	26	-12	7.81
Amygdala	**R**	**20**	**-6**	**-14**	**7.33**
Amygdala	**L**	**-22**	**-4**	**-14**	**6.47**
IFG Pars Orbitalis	R	28	30	-10	6.12
Insula	**R**	**32**	**30**	**2**	**5.57**
Fusiform Gyrus	L	-32	-32	-16	6.02
Parahippocampal Gyrus	L	-14	-28	-16	5.13
graphic - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.86**
Declive	L	-38	-70	-10	19.05
Fusiform Gyrus	R	42	-80	-10	18.41
Hippocampus	L	-22	-30	-2	11.35
Hippocampus	R	22	-30	0	13.19
Precentral Gyrus	L	-46	-4	48	9.42
Precentral Gyrus	L	-50	6	38	8.68
Precentral Gyrus	L	-42	6	32	8.47
SMA	L	-6	6	58	8.54
SMA	R	6	10	52	7.87
Precentral Gyrus	R	46	8	34	8.36

Middle Frontal Gyrus	R	50	36	24	8.31
IFG Pars Opercularis	R	54	22	30	7.91
Insula	**L**	**-30**	**30**	**-4**	**7.46**
Parahippocampal Gyrus	R	36	-6	-26	6.54
Amygdala	**L**	**-22**	**-2**	**-16**	**6.38**
Amygdala	**R**	**22**	**-4**	**-14**	**6.1**
Parahippocampal Gyrus	L	-30	-34	-16	5.94
IFG Pars Orbitalis	R	28	30	-10	5.69
Middle Temporal Gyrus	L	-54	-46	8	5.42
suffering - Rest:					
Fusiform Gyrus	R	42	-80	-10	19.19
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.1**
Occipital Lobe (Middle)	**L**	**-26**	**-96**	**8**	**18.46**
Hippocampus	R	24	-28	-2	15.59
Hippocampus	L	-22	-28	-4	14.41
Amygdala	**R**	**20**	**-6**	**-14**	**9.36**
IFG Pars Triangularis	R	52	30	26	9.05
IFG Pars Opercularis	R	46	14	32	8.54
IFG Pars Opercularis	R	52	20	34	7.88
Insula	**L**	**-30**	**28**	**0**	**8.65**
Inferior Frontal Gyrus	L	-36	20	-18	5.25
Precentral Gyrus	L	-46	-4	48	8.48
Precentral Gyrus	L	-40	8	32	8.42
IFG Pars Triangularis	L	-44	18	26	7.72
SMA	R	6	10	52	8.14
Amygdala	**L**	**-20**	**-6**	**-14**	**7.71**
Superior Temporal Gyrus	L	-52	-52	10	7.4
Insula	**R**	**30**	**32**	**-8**	**6.31**
Inferior Parietal Lobule	L	-48	-26	52	5.56
Superior Temporal Gyrus	R	48	-40	10	5.4
symbolic - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.56**
Cuneus	**L**	**-18**	**-100**	**6**	**18.61**
Lingual Gyrus	**R**	**12**	**-94**	**0**	**17.98**
Hippocampus	R	22	-28	-2	14.14
Hippocampus	L	-22	-30	-2	11.36
IFG Pars Triangularis	L	-50	22	30	8.92
IFG Pars Opercularis	L	-42	10	30	8.57
Precentral Gyrus	L	-46	-4	48	8.5
SMA	L	-4	8	56	8.77
SMA	R	6	12	52	8.72
IFG Pars Opercularis	R	54	22	32	7.68

Middle Frontal Gyrus	R	50	36	24	7.59
Precentral Gyrus	R	46	12	32	6.76
Insula	**L**	**-30**	**28**	**0**	**7.28**
IFG Pars Orbitalis	L	-36	28	-10	7.2
Inferior Parietal Lobule	L	-46	-38	54	6.19
Inferior Parietal Lobule	L	-48	-28	52	5.32
Insula	**R**	**32**	**30**	**2**	**5.2**

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute
T-value: local maxima thresholded at $p < 0.05$ FWE corrected, extent threshold $k = 10$
a-priori ROIs indicated in **BOLD.**

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Competing Interests Statement

The authors and coauthors have no competing interests to declare.

Contributorship Statement

Roger D. Newman-Norlund helped design the experiment, collected MRI data, analyze behavioral and MRI data, conducted statistical analyses and drafted the paper. James F. Thrasher oversaw the experiment, helped design the experiment, and helped draft the paper. Johann Fridriksson helped design the experiment, recruited participants, collected behavioral and MRI data and revised the draft paper. William Brixius helped collect MRI data and revised the draft paper. Brett E. Froeliger, David Hammond and Michael K. Cummings helped design the experiment and draft the paper.

Data Sharing Statement

All data collected in this experiment is located on the hard drive of the corresponding author, R.D.N. Data analysis is ongoing and the data may still be used for additional papers. After all planned papers have been submitted, the data may be made available to others, upon written request, from R.D.N.

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Neural biomarkers for assessing different types of imagery in pictorial health warning labels for cigarette packaging: A cross-sectional study.

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Abstract

Objective Countries around the world have increasingly adopted pictorial health warning labels (HWLs) for tobacco packages to warn consumers about smoking-related risks. Research on how pictorial HWLs work has primarily analyzed self-reported responses to HWLs; studies at the neural level comparing the brain's response to different types of HWLs may provide an important complement to prior studies, especially if self-reported responses are systematically biased. In this study we characterize the brain's response to three types of pictorial HWLs for which prior self-report studies indicated different levels of efficacy.

Methods Current smokers rated pictorial HWLs and then observed the same HLWs during functional magnetic resonance (fMRI) scanning. Fifty 18- to 50-year-old current adult smokers who were free from neurological disorders were recruited from the general population and participated in the study. Demo~~graphic~~graphics, smoking-related behaviors, and self-reported ratings of pictorial HWL stimuli were obtained prior to scanning. Brain responses to HWLs were assessed using fMRI, focusing on *a priori* ~~regions of interest~~ROIs.

Results Pictorial HWL stimuli elicited activation in a broad network of brain areas associated and visual processing and emotion. Participants who rated the stimuli as more emotionally arousing also showed greater neural responses at these sites.

Conclusions Self-reported ratings of pictorial HWLs are correlated with neural responses in brain areas associated with visual and emotional processing. Study results cross-validate self-reported ratings of pictorial HWLs and provide insights into how pictorial HWLs are processed.

Strengths and limitations of this study

- This is the first study to explore the relationship between self-reported ratings of pictorial HWLs and neural responses to pictorial HWLs in a large sample (N = 50) of current adult smokers.
- This paper demonstrates the amygdala is maximally activated by pictorial HWLs that depict human ~~suffering~~suffering, followed by images that depict ~~graphic~~graphic effects of smoking, followed by ~~symbolic~~symbolic images of the negative consequences of smoking.
- This paper demonstrates that neural responses to pictorial HWLs attenuate with repeated exposure in most brain regions, but that this response is different in the amygdala.
- Further research is required in order to determine i) exactly why pictorial HWLs depicting human ~~suffering~~suffering elicited such robust responses in the amygdala and ii) whether differential adaptation to ~~Symbolic~~symbolic stimuli is relevant to the creation of optimal HWLs.

INTRODUCTION

According to the World Health Organization, smoking remains the leading cause of preventable death in the Western world.(1, 2) Smoking increases the risk of many non-communicable diseases both in smokers and in those who breathe second hand smoke.(3)[3] To help prevent tobacco use and its consequences, the World Health Organization Framework Convention on Tobacco Control (WHO FCTC) has recommended including prominent, pictorial health warning labels (HWLs) on tobacco packaging to communicate the adverse effects of smoking to consumers and to discourage smoking.(2)Experimental and observational research indicate that HWLs with pictorial imagery are *more* effective than text-only HWLs in both promoting smoking cessation and preventing the initiation of smoking behavior.(4-7)[4–7] A

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key advantage of pictorial HWLs is likely due to their ability to elicit stronger emotional responses than text-only HWLs.(8)

The increasing adoption of pictorial HWLs around the world has created a critical need for research designed to i) evaluate the relative effectiveness of different types of HWL content and ii) explain why some HWL content appears more effective than other content. Such research should guide the selection of HWL content, including the rotation of new HWL content over time. Some experimental research has found the self-reported effectiveness of pictorial HWLs is highest when it contains ~~graphic~~graphic images that depict the physical effects of smoking, followed by imagery of personal ~~suffering~~suffering (usually including a face), and finally by ~~symbolic~~symbolic representations of smoking effects that use abstract imagery or symbols.(9-12)These findings are consistent with some observational studies indicating that ~~graphic~~graphic depictions of smoking consequences work best.(13, 14)

The *primary goal* of the current experiment was to explicitly map neural responses to HWLs that contain three different subtypes of imagery that are frequently used in tobacco control communications, including HWLs on cigarette packaging: ~~graphic~~graphic representation of physical consequences of smoking; personal ~~suffering~~suffering from smoking-related consequences; and ~~symbolic~~symbolic representations of risk. Given the visual and emotional nature of pictorial HWLs, we formulated a set of *a priori* ~~*regions of interest*~~*ROIs* (ROIs) that we expected to respond to participants' observations of HWLs, including the amygdala, insula and visual cortex. Converging evidence from numerous neuroscientific investigations confirms a prominent role for the *amygdala* in emotional processing in a number of sensory modalities.(15-19) The amygdala plays a particularly important role in the processing of visual stimuli related to threat and fear.(20-22) We expected that amygdala responses would be driven by our stimuli to

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the extent that they elicited arousal, fear and perceived threat (e.g., ~~graphic~~graphic HWL vs. ~~symbolic~~symbolic HWL). We also expected pictorial HWLs to elicit robust activity in the *insula*. This area has been linked to the experience of disgust, and strongly responds to pictures of mutilation and contamination.(23-26) Finally, based on a prior investigations of the neural response to emotional pictures, we expected the *visual association cortex* to be robustly activated by the presentation of pictorial HWLs.(27-29) We expected all three subtypes of HWLs to elicit a significant response (relative to rest) in this subset of *a priori* ~~regions of interest~~ROIs.

Our *secondary goal* was to examine the relationship between self-report data indicating that HWLs that use ~~graphic~~graphic imagery are more effective than HWLs depicting human ~~suffering~~suffering, which were in turn more effective than ~~symbolic~~symbolic HWLs. We hypothesized that the neural response in our *a priori* ~~regions of interest~~ROIs would differentiate between our three types of HWL (~~Graphic~~graphic > ~~Suffering~~suffering > ~~Symbolic~~symbolic), and that participants who rated pictorial HWL stimuli as more emotionally arousing exhibit heightened activity in these areas. In order to examine these questions, 50 current adult smokers self-reported emotional arousal of HWLs of each pictorial subtype and subsequently observed the same stimuli while their brain activity was measured using fMRI.

METHOD

Participants

Fifty adult smokers between the ages of 18 and 50 (24 females, Mean Age = 27.57) took part in this study. Participants were recruited from the general public, via fliers posted in public locations around the University of South Carolina (USC) and local newspapers. All participants were neurologically healthy smokers with normal to corrected vision. Following initial phone and online screening to confirm qualification for participation, all subjects reported to the

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McCausland Center and provided informed consent prior to MRI scanning. Following completion of the study protocol, participants were paid $100 for transportation costs related to participation in the study. The experiment was performed according to the guidelines of the Declaration of Helsinki and approved by the IRB at USC.

Pictorial HWL Stimuli

A total of 57 pictorial HWLs were used, with images drawn primarily from, based on, or considered for actual HWLs implemented in different countries (**Supplementary Material**) (6, 30, 31) Nineteen pictorial HWLs were developed for each of three pictorial styles: 1) ~~Graphic~~graphic health effect - vivid depiction of physical effects of smoking on the body; 2) Human ~~suffering~~suffering - depiction of personal experience which shows the face and could include the physical, social or emotional impact of smoking-related harm and; 3) ~~Symbolic~~symbolic – representation of message using abstract imagery or symbol. HWL textual content involved short, factual statements based on HWLs that have been implemented and used in prior research (9)Textual accompaniments addressed 13 different health topics were addressed (i.e., addiction, death, emphysema, gangrene, heart disease, lung cancer, mouth cancer, pregnancy, breast cancer, second hand smoke, strokes, throat cancer, and blindness), with some topics repeated twice within categories (emphysema, death, heart disease, lung cancer, mouth cancer, stroke) Topics and text were counterbalanced across the three pictorial styles. Importantly, the mean luminance values for pictorial HWL s did not differ between subtypes (all p's > 0.18), nor did the overall color (as measured by Red, Green or Blue color values) (all p's > 0.11).

Study Procedures

Demo~~graphic~~graphic Data

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All participants were asked a series of standard questions regarding their age, gender, income, ethnicity, and current and past use of cigarettes (**Table 1)**.

Self-reported responses to HWLs

Prior to attending the laboratory session, each participant completed a short survey and rated all 57 HWLs, which were presented online and in random order. The primary reason for collecting the self-report ratings before the fMRI experiment was to minimize respondent burden, as the fMRI protocol lasted an hour. We gauged this as a greater concern than familiarization (which could attenuate subsequent BOLD response), especially as smokers are usually exposed to HWLs many times every day. Negative emotional arousal was assessed by asking participants to rate the HWL on how much it made them afraid ("How much does this warning make you feel afraid?"). As in prior research, (9, 12) participants were also queried concerning ad effectiveness ("How effective is this warning?). For both questions, participants responded with a rating of 1 to 9, with verbal anchors at either end of the rating scale (i.e., 1 = not at all, 9 = extremely).

Smoking Status Screening

To confirm smoking status, carbon monoxide (CO) levels were measured in all participants immediately prior to scanning using a piCO+ Smokerlyzer (Bedfont Scientific, Harrietsham, England). All participants also provided saliva samples immediately prior to scanning to assess cotinine (nicotine metabolite) using liquid chromatography with Tandem Mass Spectrometry (LC-MS/MS). These assays confirmed self-reported smoking status for all participants. Participants also reported the time since last cigarette, the number of days they smoked in the last 30 days,

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and the average number of cigarettes they smoked per day during that time (**Supplementary Table 1**).

Neural response to HWLs

During 50 minutes of MRI scanning, each participant completed a single, high resolution structural scan, as well as four functional MRI task runs. Each functional run was 10 minutes and 24 seconds in duration. During the entire scanning run of four runs, each of the 57 images (19 graphic images, 19 suffering images and 19 symbolic images) was presented a total of 10 times each. These images were presented using a block design format. Each block of stimuli was 15 seconds in duration and consisted of the serial presentation of 5 images from the relevant condition (or fixation cross for Rest), separated by 1 second of fixation. A total of 40 blocks (10 graphic images, 10 suffering images, 10 symbolic images and 10 Rest) were presented during each of four functional runs, for a total of 150 HWLs per functional run (50 in each category). The 150 images within a given functional run were randomly chosen from a pool of 600 images created at the beginning of the scanning run. This pool of 600 images consisted of 10 of each individual HWL (10*19*3 = 570), with the remaining 30 being randomly chosen (10 pseudo-random choices from each category-the constraint being that they all had to be different, i.e. no repeats within this subset) (Figure 1) The order of presentation of the blocks within a given functional run was chosen from one of eight pseudo-randomly generated trial orders. These orders were constrained such that i) each condition was equally likely to follow any other condition within a certain functional run; and ii) blocks of the same trial type never occurred more than three times in a row. Each of the four functional runs was identical in duration and content with the exception of the random assignment of images from each condition to its

corresponding block. Importantly, the total time (and thus total number of brain volumes recorded) spent showing blocks of each picture type was identical to the total time spent showing Rest blocks.

In order to ensure that participants paid attention to the visual stimuli, we employed a 1-back picture recognition task. Participants were instructed to press a button when the same picture appeared twice in a row. This occurred 5 or 6 times (randomly chosen to prevent participants from assuming they were done detecting repeats within a given run) during each functional run. Placement of repeats was randomized prior to each run using Presentation's built in randomization features.

fMRI Methods

Image Acquisition

All MRI data were collected on a 3T Siemens Trio system with a 12-element head coil. The fMRI (T_2* echo planar imaging) imaging sequence included the following parameters: 320 full brain volumes collected in each of the four 10-minute, 24-second runs; 75° flip angle; time repetition (TR) = 1.95 s; time echo (TE) = 30 ms; in-plane resolution 3.30 × 3.30 mm; slice thickness = 3.0 mm (no gap); 36 axial slices collected in planes aligned parallel to the anterior commissure–posterior commissure line. To improve coregistration of images, all participants were scanned with a high-resolution T_1 MRI, which yielded a 1-mm isotropic image. This sequence had the following parameters: field of view (FOV) = 256 × 256 mm, 192 saggital slices, 9° flip angle, TR = 2250 ms, TE = 4.15 ms.

Data Preprocessing and Modelling

All fMRI data were preprocessed and analyzed using SPM8 (Wellcome Department of Cognitive Neurology, London). Standard preprocessing procedures included image realignment

(4th Degree B-Spline Interpolation), coregistration (Mean EPI aligned with T1 then parameters applied to all EPIs), normalization and spatial smoothing (Gaussian Kernel FWHM 8mm). The onsets and durations of each of the conditions of interest were modeled according to the block design described in the protocol. For our primary analysis, functional data across the four runs was modeled as a boxcar canonically convolved hemodynamic response function (duration 10 seconds). For results regarding between-run differences (i.e. neural adaptation), condition-specific activation within each functional run was modeled as a separate set of events. For all group analyses reported below, we first generated a series contrast images for each individual participant (first level models) and then entered these into random effects models and/or regression models (using SPM's built in general linear model) in order to allow for meaningful population-level inference. First eigen-variates were extracted from second-level models (for each ROI/condition/run) using the VOI toolbox in SPM 8.(32) For the multiple regression analysis between self-reported ratings and neural responses reported below, means for neural responses were calculated at the HWL level (mean values were calculated for each participant for the neural response in each ROI and for each HWL subtype). The resulting parameter estimates were used as the primary dependent variables in the statistical models reported below (i.e. ANOVA and regression analyses).

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RESULTS

Behavioral Performance

Population Variables

Our Participants in the current study were equally spilt with respect to gender (52% Male, 48% Female) and predominantly white (74%, 24% African American, 2% other). The majority of participants (55%) had at least some post-high school education, and were low-income. At the

time of scanning, the group's CO levels were 18.74 ppm and cotinine was measured at 207.48 ng/mm confirming that all participants were active smokers. Furthermore, the average participant smoked 18.74 cigarettes per day, and reported having smoked on 28.32 out of the previous 30 days.

Self-reported Ratings of HWLs

Differences in self-reported emotional arousal across the three stimulus types (~~Graphic~~graphic, ~~Suffering~~suffering, ~~Symbolic~~symbolic) was assessed using one-way within subjects ANOVA, $F(1.44,70.53) = 121.01$, $p < 0.001$. A one-way within subjects ANOVA using perceived effectiveness as a dependent variable and stimulus-type (graphic, suffering, symbolic) as the dependent variable was also significant, $F(1.54,75.27) = 133.27$, $p < 0.001$. For both ANOVAS, post-hoc pair-wise comparisons revealed significant differences between ratings of graphic and suffering stimuli, as well as between ratings of suffering and symbolic stimuli (all p's < 0.01).

Responses to the emotional arousal and perceived effectiveness questions were highly correlated for the ~~Graphic~~graphic ($r(49) = .87$), ~~Suffering~~suffering ($r(49) = .90$) and ~~Symbolic~~symbolic ($r(49) = .90$) stimuli. Because ratings of emotionality were the most relevant for interpretation of our results, we focus on those scores in our analysis section. We would like to note that we did perform the same analyses using perceived effectiveness and obtained a similar pattern of results. (**Figure 2**)

fMRI One-back Task:

One-back task performance data was collected from a total of 176 out of 200 possible fMRI scanning runs (50 participants, with 4 runs per person). Data from 24 of the runs was lost due to experimenter error. We did not exclude the imaging data from these participants as we did

monitor the participants' error rates online and ensure they were paying attention (they were just not recorded). A one-way ANOVA using *error rate* as the dependent variable with run as the factor was not significant, F (3,162) = 1.003, p = 0.393. Moreover, post-hoc comparison of all possible run pairings failed to reveal any significant differences in 1-back performance between n any two runs (all p's > 0.33).

fMRI Response

Primary fMRI Outcomes

Main Effects of HWL Type

In order to isolate cortical networks activated by the presentation of each type of pictorial HWL, we computed a series of contrasts designed to test for the main effects of each of the three stimulus types (~~Graphic~~graphic, ~~Suffering~~suffering, and ~~Symbolic~~symbolic). Specifically, we computed the following contrasts: ~~Graphic~~graphic-Rest, ~~Suffering~~suffering-Rest and ~~Symbolic~~symbolic-Rest (thresholded at p < 0.05 and corrected for family-wise error (FWE)). Observation of pictorial HWL stimuli elicited a significant neural response in a broad network of brain areas including our *a priori* ~~regions of interest~~ROIs (the amygdala, insula, and visual association cortex) as well as a number of other brain areas including the frontal gyrus (inferior, middle, medial, and superior aspects), temporal gyrus (middle and superior), parietal lobe (inferior), supplementary motor area, parahippocampal gyrus, and thalamus. The results of this analysis are listed in **Table 1** and displayed ~~graphic~~graphically in **Figure 3**.

Comparison of HWL-elicited Activation in a priori ROIs

We performed additional analyses in order to identify brain areas whose response properties showed the same pattern as participants' self-reported evaluations of the experimental

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stimuli in each group (~~Graphic~~graphic > ~~Suffering~~suffering > ~~Symbolic~~symbolic). Accordingly, we performed ROI analyses on our *a priori* ~~regions of interest~~ROIs including the amygdala, insula and secondary visual cortex. ~~Regions of interest~~ROIs within the visual association cortex, amygdala and insula were created based on peak activations observed in the contrast comparing the brain's response to all conditions to rest ([~~Graphic~~graphic + ~~Suffering~~suffering + ~~Symbolic~~symbolic] – Rest). All ROIs were centered at the site of peak activation within a given ROI and were spherical in nature (r = 4 mm). A series of one-way within-subjects ANOVAs were used to evaluate neural responses patterns (for ~~Graphic~~graphic, ~~Suffering~~suffering and ~~Symbolic~~symbolic stimuli) within our ROIs. These ANOVAs were significant in the left amygdala, $F(2,98) = 14.59$, $p < 0.001$, right amygdala, $F(2,98) = 21.60$, $p < 0.001$, left insula, $F(2,98) = 4.42$, $p < 0.05$, and visual association cortex, $F(2,98) = 22.69$, $p < 0.001$. As with the behavioral data, we conducted post-hoc pairwise comparisons (all significant results were $p < 0.05$, Bonferroni corrected). In the left amygdala we observed a significant difference between responses in the ~~Graphic~~graphic and ~~Symbolic~~symbolic conditions, as well as in the ~~Suffering~~suffering and ~~Symbolic~~symbolic conditions. In the right amygdala all pair-wise comparisons were significant. In the left amygdala and the visual association cortex, responses to ~~Graphic~~graphic and ~~Symbolic~~symbolic stimuli were significantly different, as were responses to ~~Graphic~~graphic and ~~Suffering~~suffering stimuli. The results of these analyses are shown ~~graphic~~graphically in **Figure 4, A.** We also conducted whole-brain analyses for the following direct comparisons between conditions: graphic > symbolic : symbolic > graphic (**Supplementary Table 2**), suffering > symbolic : symbolic < suffering, (**Supplementary Table 3**) and suffering > graphic : graphic > suffering (**Supplementary Table 4**).

Secondary fMRI Outcomes

Correlation Between Self-Reported Ratings and Neural Response

We ran a series of targeted correlations to determine whether there was a relationship between individual ratings of pictorial HWLs of specific subtypes and the BOLD signal elicited by their presentation. For the ~~graphic~~graphic stimuli, we conducted an SPM multiple regression analysis using individual contrast images for the ~~Graphic~~graphic-Rest condition as the dependent variable and mean self-reported arousal ratings for the ~~Graphic~~graphic HWLs as the independent variable (thresholded at $p < 0.001$, 5 voxel extent). Similar regression analyses were conducted to examine the correlation between HWL ratings and BOLD signal in the ~~Suffering~~suffering and ~~Symbolic~~symbolic conditions. In all three analyses, activation in the right visual association cortex (XYZ_{mni} = -18, -92, 20, XYZ_{mni} = -20, -88, 12, and XYZ_{mni} = -14, -92,12 respectively) was positively correlated with mean ratings of the pictorial HWLs (all r(49)'s > .48) (**Figure 5**). For ~~graphic~~graphic and ~~suffering~~suffering HWLs additional positive correlations were found at sites in the right precentral gyrus (XYZ_{mni} = 44,4,40), r(49) = .45 and r(49) = .42 respectively. For ~~symbolic~~symbolic HWLs there was an additional positive correlation between HWL ratings and activation in the left inferior frontal gyrus (XYZ_{mni} = -52,16,30), r(49) = .37).

Exploratory Analysis of BOLD Signal Adaptation

In addition to examining the main effects of stimulus type, we also conducted a series of 3 (Stimulus) x 4 (Run) repeated measures ANOVAs (one for each ROI) in order to explore possible BOLD signal adaptation to our three stimuli types across the four fMRI runs. The main effect of run was significant for the left insula, $F(3,138) = 11.40$, $p < 0.001$, right insula $F(3,138) = 3.19$, $p < 0.05$, and visual association cortex, $F(3,138) = 15.43$, $p < 0.001$, and nearly significant in the left amygdala, $F(3,138) = 2.66$, $p = 0.074$. There was a significant interaction between Stimulus and Run in both the left amygdala, $F(6,276) = 2.28$, $p < 0.05$, and right

amygdala, $F(6,276) = 2.15$, $p < 0.05$. These results are shown split by run (in order to visualize adaptation) in **Figure 4, B**.

DISCUSSION

Self-reported Ratings of Pictorial HWLs

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Results from the current study were generally consistent with prior research using self-reported responses to HWL stimuli. This research consistently indicates that smokers report stronger responses to HWLs with graphic imagery than to symbolic imagery. (10, 11, 31, 33, 34, 37) Results suggesting the greater impact of imagery of suffering than graphic imagery are not necessarily inconsistent with this research. Indeed, a number of the suffering images included graphic elements, and HWLs that combine the two may be may be most effective. (31) Nevertheless, as for self-report research, future fMRI research is needed to determine whether neural responses predict meaning behavioral change (i.e., quitting smoking) or perceptual change (e.g., better understanding of risks, particularly among youth). In general, however, this study suggests that fMRI and self-report produce similar results. One possible concern with the present results is that we did not confirm our specific sample of participants considered each pictorial HWL to belong to one category or another. Future research may consider asking participants to sort pictorial HWLs into categories to address this concern.

Main Effects of HWL Type

The primary goal of the current experiment was to assess neural responses to the presentation of different types of pictorial HWLs that governments have considered for implementation. In general, observation of pictorial HWLs activated large-scale neural networks including the hippocampus, fusiform gyrus, precentral gyrus, supplementary motor area, pars Triangularis, pars opercularis, pars orbitalis and fusiform gyrus. Based on prior literature

mapping the brain's response to vivid graphic images, we expected all three types of HWLs to elicit activation in the amygdala, the insula and the visual association cortex. Our results are consistent with this literature in that all subtypes of pictorial HWLs used in the current study elicited activation at sites in all three of these areas.

The present study explicitly measured neural responses to observation of pictorial HWLs in a population of confirmed cigarette smokers. Results indicated that pictorial HWLs of all types elicited activation in areas associated with visual processing, as well as the processing of fear and disgust. Activation at sites in the inferior frontal gyrus/precentral gyrus, visual cortex, and to a lesser extent the insula, showed a pattern for strength of response by pictorial stimulus type (i.e., ~~Graphic~~graphic > ~~Suffering~~suffering > ~~Symbolic~~symbolic) that was the same as was found for participants' self-reported ratings of the fear elicited by the stimuli. However, amygdala responses appeared more complex, and it responded maximally to pictorial HWLs depicting human ~~suffering~~suffering, perhaps due to its involvement in empathetic responses (see below). Previous experimental research has found that HWL imagery that combines human ~~suffering~~suffering with ~~graphic~~graphic imagery is rated as more effective than either imagery type alone.(9) In many cases the ~~suffering~~suffering imagery used in our study included ~~graphic~~graphic elements, and that combination may most effectively promote amygdala response. Finally, for all pictorial HWLs, participants that perceived the pictorial HWLs as particularly effective showed heightened activation in the visual association cortex.

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~~The primary goal of the current experiment was to measure the neural response to presentation of pictorial HWLs. Based on prior literature mapping the brain's response to vivid graphic images, we expected the more graphic HWLs to elicit activation in the amygdala, and insula. Our results are consistent with this literature in that all subtypes of pictorial HWLs used in the current study elicited activation at sites in the amygdala, the insula and the visual association cortex.~~

Comparison of HWL-elicited Activation in *a priori* ROIs

Visual Association Cortex

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~~***Region of Interest Analysis***~~

~~A secondary goal of this experiment was to examine the relationship between self-reported ratings of pictorial HWLs with brain data.~~ We expected that responses in regions associated with visual and emotional processing would mirror self-reported ratings of the stimuli (i.e. ~~Graphic~~**graphic** > ~~Sufferings~~**suffering** > ~~Symbolics~~**symbolic**). Results from our ROI analysis were partially consistent with this prediction. Activity in the right visual association cortex did

scale in the same manner as self-reported ratings. The more vivid/~~graphic~~**graphic** nature of certain subtypes of pictorial HWLs may be responsible for the differences we observed in the visual cortex. Images in the ~~Graphic~~**graphic** condition contained more gory/bloody elements than those in any of the other two conditions~~.~~**;** the images in the suffering condition contained a moderate amount of these elements; and images in the symbolic condition contained the least of these elements. We speculate that these negatively valenced elements, which were particularly arousing, may have increased signal in visual areas via afferent projections from the amygdala. It is well established that the amygdala, a key neural pathway for responses to graphic imagery, projects to both primary and secondary visual cortices.(38) It is particularly unlikely that heightened activation in the visual association cortex was caused by differences in low-level features of the images. Neither luminance nor color values for HWL stimuli were significantly different across the three HWL subtypes. Additionally, in at least one previous experiment examining the impact of arousing visual stimuli on visual cortex activity, differences in eye movements did not account for the observed patterns of activation.(28) Therefore it is unlikely that the effects we report were due to differential eye movements.

Amygdala

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While responses in the visual association area and insula were consistent with self-reported ratings, activation patterns observed in amygdala were not. Unexpectedly, the amygdala was most robustly activated by suffering HWLs, followed by graphic HWLs, and finally symbolic HWLs. ~~**and the images in the Suffering condition contained a moderate amount of these elements. It is well established that the amygdala, a key neural pathway for responses to graphic imagery, projects to both primary and secondary visual cortices.(33) It is unlikely that this activation was caused by differences in low-level**~~

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~~features of the images because luminance and color values were not significantly different for the three HWL subtypes. Additionally, in at least one previous experiment examining the impact of arousing visual stimuli on visual cortex activity, differences in eye movements did not account for the observed patterns of activation.(28) Therefore it is unlikely that the effects we report were due to differential eye movements.~~

~~While responses in the visual association area and insula were at minimal consistent with self-reported ratings, activation patterns observed in amygdala were not. Surprisingly, the amygdala was most robustly activated by Suffering HWLs, followed by Graphic HWLs, and finally Symbolic HWLs.~~ **As noted in the introduction, the amygdala has been shown to be responsive to arousing stimuli, and fear-evoking stimuli appear to be particularly potent at activating this brain structure. One possibility, then, is that the HWLs that depict personal ~~sufferingsuffering~~ from smoking-related outcomes are best at eliciting fear. However, this is inconsistent with the self-reported data, which indicated that ~~Graphiegraphic~~ HWLs elicited maximal fear responses. A more parsimonious explanation for this finding is that the relative hyper-activation observed for HWLs with ~~Sufferingsuffering~~ imagery was due to the presence of human faces in the stimuli (all 19 ~~Sufferingsuffering~~ HWLs contained human faces). Lesion, single-cell and whole brain neuroimaging experiments are consistent with the idea that the amygdala is a key component of the face-perception network.(18, 34-39) The amygdala may even process fearful facial stimuli in the absence of conscious processing.(40, 41)** Hence, the inclusion of faces may be particularly important to maintaining arousal-inducing responses under conditions of repeated exposure, as is typically the case with HWLs. Indeed, recent evidence suggests that sustained responses to repeated presentation of emotional faces may be particularly dependent on

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the amygdala.(47) It is also important to note that some of the suffering images (4 of 19) portrayed visible body damage, and thus suffering imagery was not entirely distinct from graphic imagery used in the current experiment, and research based on self-reported ratings indicated that this combination produces the strongest ratings.(31) To better isolate any differential effects of these two image types and the interaction between them, future studies should use imagery that more clearly falls into one category, the other, or both. Another possible explanation for the increased relative amygdala activation observed in the suffering condition relates to stimulus salience (an index of stimulus salience). Studies have demonstrated a strong link between amygdala activation and stimulus salience.(48, 49)

While these results could have implications for the optimization of HWLs, further experiments are necessary to evaluate the predictive validity of fMRI. Future research should aim to separate out the effects of emotionality, salience and human faces by integrating additional conditions (such as neutral images with and without faces). Based on research demonstrating the that BOLD signal in the amygdala is a predictor of subsequent quitting behavior (50) (as is BOLD signal in the medial prefrontal cortex (51, 52)), future prospective studies should examine the extent to which amygdalar BOLD response to the three types of HWLs discussed in the current paper predict changes in smoking behavior or, among youth, perceptions about smoking-related risks. Little research has been conducted with youth before they start smoking, and the strongest effects of HWLs may be due to enhancing aversion for smoking as opposed to changing the behaviors of addicted smokers.

Secondary fMRI Outcomes

Correlation Between Self-Reported Ratings and Neural Response

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An important goal of the present study was to cross-validate self-reported ratings of pictorial HWLs and brain activity recorded during the observation of the same stimuli. This paper is the first to report such results for smoking HWL stimuli. Regarding correlations between self-reported ratings of HWL stimuli and neural activity in our three *a priori* ROIs, only the visual cortex was significant (with the amygdala being nearly significant at p=0.07). We also report significant correlations between behavioral ratings and two additional areas, the junction of the right precentral and inferior frontal gyrus, and the left inferior frontal gyrus pars opercularis.

Visual Association Cortex~~*Faces may be particularly important under conditions of repeated exposure, as with HWLs, as we may be drawn to faces even after repeated exposure, whereas we may be less drawn to graphic bodily harm. Some of the suffering images (4 of 19) portrayed visible body damage, and so Suffering imagery was not entirely distinct from graphic imagery used. To better isolate any differential effects of these two image types and the interaction between them, future studies should use imagery that more clearly falls into one category, the other, or both. Another possible explanation for the increased relative amygdala activation observed in the Suffering condition relates to stimulus salience. Studies have demonstrated a strong link between amygdala activation and stimulus salience.(42, 43) In the context of the current experiment, it may be that images depicting smoking-related suffering were particularly salient to current smokers. While this could have implications for the optimization of HWLs, further experimentation is necessary to evaluate this hypothesis. Future research should aim to separate out the effects of emotionality, salience and human faces by integrating additional conditions (such as neutral images with and without faces). Based on research demonstrating the that BOLD signal in the amygdala is a predictor of subsequent quitting behavior (44) (as is~~

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~~*BOLD signal in the medial prefrontal cortex (45, 46)), it might be useful to conduct future prospective studies that examine the extent to which amygdalar BOLD response to the three types of HWLs discussed in the current paper predict changes in smoking behavior.*~~

An important goal of the present study was to cross-validate self-reported ratings of pictorial HWLs and brain activity recorded during the observation of the same stimuli. This paper is the first to report such results for cigarette HWL stimuli. Regarding correlations between self-reported ratings of HWL stimuli and neural activity in our three *a priori* ROIs, only the visual cortex was significant (with the amygdala being nearly significant at p=0.07). We also report significant correlations between behavioral ratings and two additional areas, the junction of the right precentral and inferior frontal gyrus, and the left inferior frontal gyrus pars opercularis.

Insula and Amygdala~~*To the extent that HWL effectiveness depends on enduring emotional responses, neural adaptation to repeated exposure is an important issue to consider. Our exploratory, post hoc analysis of region specific adaptation revealed that, in the majority of our regions of interest, BOLD response decreased as a function of repeated exposure to all HWLs. Interestingly, we observed a significant deviation from this pattern in the left and right amygdala. While activation associated with observation of Graphic and Suffering images consistently decreased across the four sessions, activation patterns associated with observation of Symbolic images were less consistent (**Figure 4, B**). It is tempting to speculate that participants did not adapt (neutrally speaking) to repeated presentation of Symbolic stimuli in the same way they adapted to images in the Suffering and Symbolic categories. The abstract nature of these stimuli may have necessitated additional exposure in order to fully process their meaning, and this may account for the observed findings. These data should be interpreted*~~

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~~*cautiously as repeated exposure to HWLs during three, 10-minute scanning sessions may not accurately mimic repeated exposure to HWLs as it exists in real-life (temporally spread out, situation specific, craving-state specific, etc.). Further scrutiny of neural adaptation across repeated sessions or repeated days could isolate differences in neural adaptation. If these neural responses can be linked to changes in smoking behavior, public health could be positively impacted.*~~

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~~Relationship Between Neural Measures and Self-Report Data~~

~~**An important goal of the present study was to cross-validate self-reported ratings of pictorial HWLs and brain activity recorded during the observation of the same stimuli. This paper is the first to report such results for smoking HWL stimuli. In general, our correlational data indicate that participants who rated pictorial HWL stimuli (within each category – as opposed to between categories) as more emotionally arousing showed higher activation of the visual association cortex when viewing the stimuli. This finding is consistent with previous reports demonstrating that activity in the visual cortex is particularly robust during the presentation of emotionally arousing visual stimuli, perhaps due to reentrant enhancement of V2 activity being driven by motivational processes that heighten input from the amygdala. (27, 29, 47)[27–29]**~~

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~~**We also observed an unexpected correlation between self-report ratings and activity at the junction of the right precentral gyrus and inferior frontal gyrus (pars opercularis). Given the location of the activation in the RH (as opposed to the LH which is traditionally associated with such language functions), it is unlikely that heightened responses reflect increased reliance on language. This site is considered to be part of the human mirror neuron system (MNS) and thought to interact with the amygdala and insula when we**~~

~~establish a link between the actions/emotions/intentions of others and our own actions.(48)[49] These stimuli may have been particularly effective at eliciting the types of interpersonal comparisons and or emotions (i.e. empathy) that individuals typically make when seeing the negative effects of their own behaviors in others.(46, 49-51)[49-52] Another possible explanation for the significant correlation we observed between right IFG activity and self-reported ratings is that more emotionally arousing stimuli required greater emotion regulation on the part of the observer. This is consistent with studies reporting recruitment of the right IFG during tasks that require the inhibition of emotions. (52-54)~~

~~Finally, we observed a significant relationship between activity in the left inferior frontal gyrus (BA 44) and self-report ratings of the symbolic stimuli. This area has traditionally been associated with language processing and is active during both overt (i.e. spoken) and covert (i.e. silent) speech.(55-59)[53-57] It is not surprising that symbolic stimuli would utilize language processes. Stimuli of this subtype were the most abstract and likely evoked covert speech during the interpretation process. These data suggest that the Symbolic HWL stimuli that maximally engage language processes are likely to be rated as more arousing than those that do not. If symbolic stimuli are too abstract/confusing to easily verbalize (covertly), then they may be interpreted as more fear eliciting. The involvement of language areas during HWL processing could be the topic of future experiments that assess verbalization during presentation of HWLs of all types.~~

Surprisingly, we did not observe a significant correlation between BOLD signal in the insula or amygdala and self-reported ratings of arousal. However, the correlation between BOLD signal ~~. While we did not find significant correlations between amygdala activity and self-~~

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~~reported ratings of arousal (as might be expected), the correlation between BOLD signal in~~ the right amygdala and self-reported responses in both ~~Graphic~~**graphic** (r(49) = .21, p = 0.07 one-tailed) and ~~Suffering~~**suffering** (r(49) = .20, p = 0.08 one-tailed) conditions was nearly significant~~, and in the predicted direction~~. This failure to reach statistical significance may be due to a number of reasons. One possibility is that the amygdala's response to the emotional stimuli was blunted by the inclusion of text in the HWLs used in the present study. This interpretation is consistent with a comprehensive meta-regression analysis of imaging studies on amygdala activation, which found that presence of language in the stimulus was associated with reduced amygdala activation (as well as greater left lateralization relative to baseline).(54) While the inclusion of text in graphic warning labels has traditionally been justified in terms of added information content (text adds information otherwise not present), it may also be important to examine possible emotional 'blunting' effects that its inclusion may have. Future brain imaging studies might explore this possibility by simultaneously monitoring brain activity and gaze behavior. A better understanding of the how people process graphical and textual elements of HWLs, and how attention to one or the other affects neural processing, particularly after repeated HWL exposure that simulates naturalistic exposure conditions, may help inform the design of future HWLs. ~~It is useful to consider why this correlation might have failed to reach statistical significance. One possibility for this negative finding is that the amygdala's response to the emotional stimuli was blunted by the inclusion of text in the HWLs used in the present study. This interpretation is consistent with a comprehensive meta-regression analysis of imaging studies reporting amygdala activation which found that presence of language in the stimulus was associated with reduced amygdala activation (as well as greater left lateralization relative to baseline).(60) This finding is particularly interesting in light of trends towards the adoption~~

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~~image based HWLs. While the inclusion of text in graphic warning labels has traditionally been justified in terms of added information content (text adds information otherwise not present), it may also be important to examine possible emotional 'blunting' effects that inclusion of text may have.~~

Junction of Right Precentral Gyrus and Inferior Frontal Gyrus

We also observed an unexpected correlation between self-report ratings and activity at the junction of the right precentral gyrus and inferior frontal gyrus (pars opercularis) for suffering HWLs only. Given the location of the activation in the RH (as opposed to the LH which is traditionally associated with such language functions), it is unlikely that heightened responses reflect increased reliance on language. This site is considered to be part of the human mirror neuron system (MNS) and thought to interact with the amygdala and insula when we establish a link between the actions/emotions/intentions of others and our own actions.(48)[49] **One possible explanation for this finding is that suffering stimuli may have been particularly effective at eliciting the types of interpersonal comparisons and or emotions (i.e. empathy) that individuals typically make when seeing the negative effects of their own behaviors in others.(52, 56-58)** Another possible explanation for the significant correlation we observed between right IFG activity and self-reported ratings is that more emotionally arousing stimuli required greater emotion regulation on the part of the observer. This is consistent with studies reporting recruitment of the right IFG during tasks that require the inhibition of emotions. (52-54)

Inferior Frontal Gyrus, Pars Opercularis

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Finally, we observed a significant relationship between activity in the left inferior frontal gyrus (BA 44) and self-report ratings of the symbolic stimuli. This area has traditionally been associated with language processing and is active during both overt (i.e. spoken) and covert (i.e. silent) speech.(55-59)[53–57] It is not surprising that symbolic stimuli would utilize language processes. Stimuli of this subtype were the most abstract and likely evoked covert speech during the interpretation process. The involvement of language areas during HWL processing could be the topic of future experiments that assess verbalization during presentation of HWLs of all types. While it is reasonable to expect that activation of language areas during HWL processing (an indirect measure of covert verbalization) may be related to subsequent behavioral change, future studies will need to address this possibility.

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~~*Future brain imaging could further*~~ *Exploratory Analysis of BOLD Signal Adaptation*

To the extent that HWL effectiveness depends on enduring emotional responses, neural adaptation to repeated exposure may be an important issue to consider. Our exploratory, post-hoc analysis of region-specific adaptation revealed that, in the majority of our ROIs, BOLD response decreased as a function of repeated exposure to all HWLs. Interestingly, we observed a significant deviation from this pattern in the left and right amygdala. While activation associated with observation of graphic and suffering images was higher overall, it consistently decreased across the four runs, whereas activation patterns associated with observation of symbolic images was lower and less consistent (Figure 4, B). Hence, participants may not have adapted (neurally speaking) to repeated presentation of symbolic stimuli in the same way they adapted to images in the suffering and symbolic categories. The abstract nature of symbolic stimuli may have required additional exposures in order to more fully process their meaning, and this may account for the observed findings. These data should be interpreted cautiously, however, as repeated exposure to

HWLs during three, 10-minute scanning runs is unlikely to accurately mimic repeated exposure to HWLs as in real-life, which is temporally spread out, situation specific, and associated with cravings and branding imagery that weakens HWL effects. Future research should more directly examine the relationship between the strength of brain activity elicited by specific subtypes of pictorial HWLs after repeated exposures to HWLs, including more naturalistic exposures that allow for adaptation and habituation.

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Possible Implications for Public Health Policy

Understanding how the brain responds to HWLs can inform the optimal development of HWLs. For example, studies on smokers' neural responses to different types of anti-smoking ads has found that the strength of neural responses elicited by health messaging predicts subsequent individual-level behavioral change as well as the population-level efficacy of different types of ads responses to ads once they are aired in media campaigns (52) While the current study does not report on behavioral change, future research should. Furthermore, if predictive validity of these methods is established, they could be used to assess the behavioral effects of other types of HWL content. The cost-effectiveness of fMRI compared to self-report studies should also be assessed, particularly if they provide consistent results, as we have found here. Data regarding neural adaptation caused by repeated exposure to pictorial HWLs is could also be important in terms of informing the creation of HWLs designed for maximum long-lasting impact. Arguably, HWLs will only be effective to the extent that they continue to elicit responses from the consumer. Knowing whether or not consumers differentially adapt to different types of HWL content will allow for choice of HWLs that are most likely to discourage smoking.

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Study Limitations

Understanding how the brain responds to HWLs can inform the optimal development of HWLs. For example, studies on smokers' neural responses to different types of anti-smoking ads has found that the strength of neural responses predicts subsequent individual-level cessation behavior(52) as well as population-level cessation attempts (i.e., volume of calls to quitlines) due to different types of ads once they are aired in media campaigns.(67) While the current study does not report on behavioral change, future research should. Furthermore, if the predictive validity of these methods is established, they could be used to evaluate the efficacy of a range of HWL content and presentation styles. The cost-effectiveness of fMRI compared to self-report studies should also be assessed, particularly if they provide consistent results, as we have found here. Data regarding neural adaptation caused by repeated exposure to pictorial HWLs could also be important in terms of informing the creation of HWLs designed for maximum long-lasting impact. HWLs are likely to be most effective if they elicit consumer responses over time. Indeed, the motivation to process messages changes over time, as does the motivation to quit smoking (68) and HWLs effects may become more potent as these motivations change. Knowing more about the process of adaptation to different types of HWL content, including potential differences in the processes of adaptation across diverse groups, may help with designing HWLs that are most likely to discourage smoking.

~~Summary~~General Conclusion

The present study examined self-reported and neural responses to pictorial HWL stimuli of three different types in a population of current adult smokers. Pictorial HWLs elicited robust responses in a broad network of brain sites including those associated with image interpretation (visual association cortex) and emotion (amygdala and insula). Moreover, activation in visual,

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premotor, inferior frontal and to a lesser extent the insular areas, varied in the same manner as self-reported ratings of the stimuli. We report a robust relationship between self-reported ratings of arousal and neural responses, which is important considering that self-reported data can be subject to bias. Our exploratory, post hoc analysis of BOLD signal attenuation across scanning runs revealed differences in the patterns of neural adaptation for different types of HWLs that may be relevant to the optimization of future HWLs. Gaining a better grasp of the relationship between self-reported ratings of HWLs, neural responses elicited by HWLs, and the effectiveness of HWLs should be an important goal of future research.

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NEURAL RESPONSE TO HEALTH WARNING LABELS

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Table 1.

region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
ALL - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**21.62**
Fusiform Gyrus	R	42	-80	-10	19.48
Calcarine	R	12	-94	0	19.02
Hippocampus	R	20	-30	0	15.8
Hippocampus	L	-22	-30	-2	13.73
IFG Pars Triangularis	L	-52	24	30	9.87
Precentral Gyrus	L	-46	-4	52	9.71
Precentral Gyrus	L	-42	8	32	9.26
SMA	L	-6	8	56	8.99
SMA	R	6	10	52	8.53
IFG Pars Triangularis	R	48	24	26	8.67
IFG Pars Opercularis	R	54	22	32	8.66
Middle Frontal Gyrus	R	50	36	24	8.64
Insula	**L**	**-30**	**28**	**2**	**8.39**
IFG Pars Orbitalis	L	-34	30	-8	8.17
IFG Pars Orbitalis	L	-40	26	-12	7.81
Amygdala	**R**	**20**	**-6**	**-14**	**7.33**
Amygdala	**L**	**-22**	**-4**	**-14**	**6.47**
IFG Pars Orbitalis	R	28	30	-10	6.12
Insula	**R**	**32**	**30**	**2**	**5.57**
Fusiform Gyrus	L	-32	-32	-16	6.02
Parahippocampal Gyrus	L	-14	-28	-16	5.13
graphic - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.86**
Declive	L	-38	-70	-10	19.05
Fusiform Gyrus	R	42	-80	-10	18.41
Hippocampus	L	-22	-30	-2	11.35
Hippocampus	R	22	-30	0	13.19
Precentral Gyrus	L	-46	-4	48	9.42
Precentral Gyrus	L	-50	6	38	8.68
Precentral Gyrus	L	-42	6	32	8.47
SMA	L	-6	6	58	8.54

SMA	R	6	10	52	7.87
Precentral Gyrus	R	46	8	34	8.36
Middle Frontal Gyrus	R	50	36	24	8.31
IFG Pars Opercularis	R	54	22	30	7.91
Insula	**L**	**-30**	**30**	**-4**	**7.46**
Parahippocampal Gyrus	R	36	-6	-26	6.54
Amygdala	**L**	**-22**	**-2**	**-16**	**6.38**
Amygdala	**R**	**22**	**-4**	**-14**	**6.1**
Parahippocampal Gyrus	L	-30	-34	-16	5.94
IFG Pars Orbitalis	R	28	30	-10	5.69
Middle Temporal Gyrus	L	-54	-46	8	5.42
suffering - Rest:					
Fusiform Gyrus	R	42	-80	-10	19.19
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.1**
Occipital Lobe (Middle)	**L**	**-26**	**-96**	**8**	**18.46**
Hippocampus	R	24	-28	-2	15.59
Hippocampus	L	-22	-28	-4	14.41
Amygdala	**R**	**20**	**-6**	**-14**	**9.36**
IFG Pars Triangularis	R	52	30	26	9.05
IFG Pars Opercularis	R	46	14	32	8.54
IFG Pars Opercularis	R	52	20	34	7.88
Insula	**L**	**-30**	**28**	**0**	**8.65**
Inferior Frontal Gyrus	L	-36	20	-18	5.25
Precentral Gyrus	L	-46	-4	48	8.48
Precentral Gyrus	L	-40	8	32	8.42
IFG Pars Triangularis	L	-44	18	26	7.72
SMA	R	6	10	52	8.14
Amygdala	**L**	**-20**	**-6**	**-14**	**7.71**
Superior Temporal Gyrus	L	-52	-52	10	7.4
Insula	**R**	**30**	**32**	**-8**	**6.31**
Inferior Parietal Lobule	L	-48	-26	52	5.56
Superior Temporal Gyrus	R	48	-40	10	5.4
symbolic - Rest:					
Lingual Gyrus	**R**	**24**	**-90**	**-6**	**19.56**
Cuneus	**L**	**-18**	**-100**	**6**	**18.61**
Lingual Gyrus	**R**	**12**	**-94**	**0**	**17.98**
Hippocampus	R	22	-28	-2	14.14
Hippocampus	L	-22	-30	-2	11.36
IFG Pars Triangularis	L	-50	22	30	8.92
IFG Pars Opercularis	L	-42	10	30	8.57
Precentral Gyrus	L	-46	-4	48	8.5
SMA	L	-4	8	56	8.77

SMA	R	6	12	52	8.72
IFG Pars Opercularis	R	54	22	32	7.68
Middle Frontal Gyrus	R	50	36	24	7.59
Precentral Gyrus	R	46	12	32	6.76
Insula	**L**	**-30**	**28**	**0**	**7.28**
IFG Pars Orbitalis	L	-36	28	-10	7.2
Inferior Parietal Lobule	L	-46	-38	54	6.19
Inferior Parietal Lobule	L	-48	-28	52	5.32
Insula	**R**	**32**	**30**	**2**	**5.2**

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute
T-value: local maxima thresholded at $p < 0.05$ FWE corrected, extent threshold $k = 10$
a-priori ROIs indicated in **BOLD.**

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Competing Interests Statement

The authors and coauthors have no competing interests to declare.

Contributorship Statement

Roger D. Newman-Norlund helped design the experiment, collected MRI data, analyze behavioral and MRI data, conducted statistical analyses and drafted the paper. James F. Thrasher oversaw the experiment, helped design the experiment, and helped draft the paper. Johann Fridriksson helped design the experiment, recruited participants, collected behavioral and MRI data and revised the draft paper. William Brixius helped collect MRI data and revised the draft paper. Brett E. Froeliger, David Hammond and Michael K. Cummings helped design the experiment and draft the paper.

Data Sharing Statement

All data collected in this experiment is located on the hard drive of the corresponding author, R.D.N. Data analysis is ongoing and the data may still be used for additional papers. After all planned papers have been submitted, the data may be made available to others, upon written request, from R.D.N.

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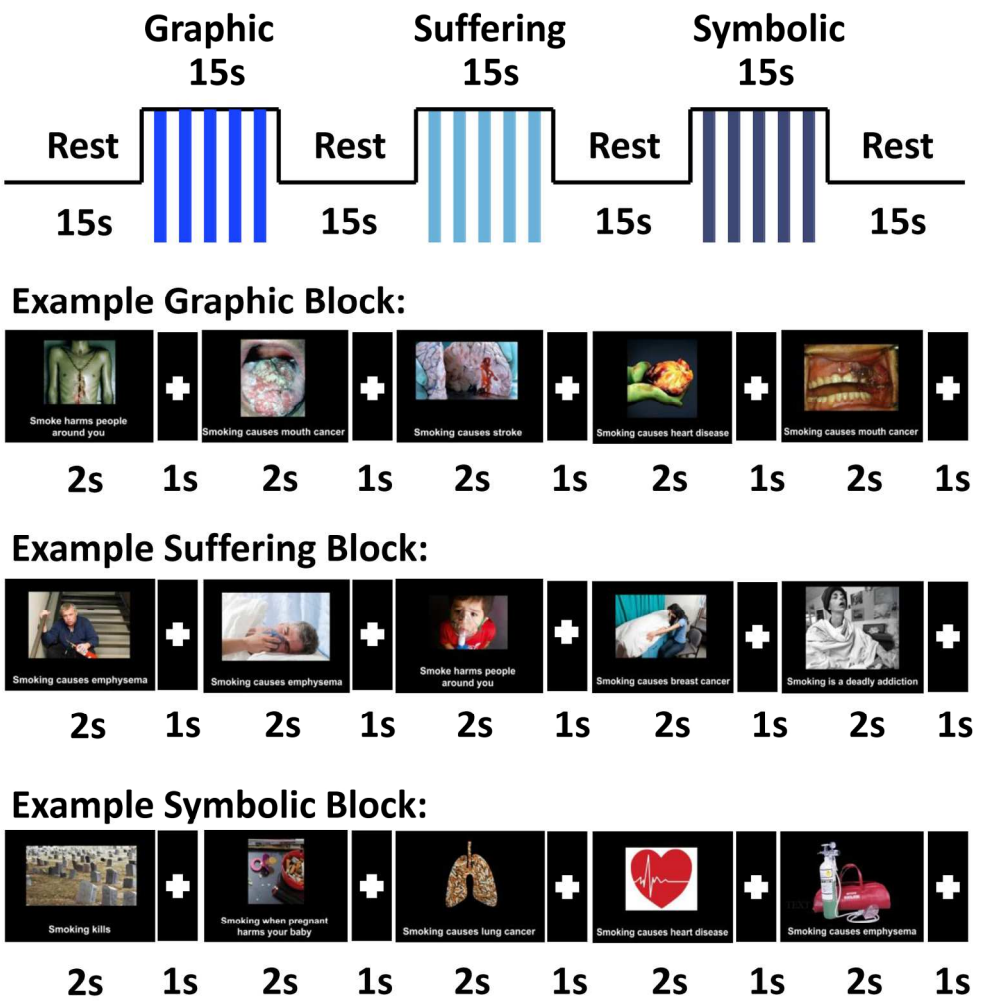


Fig. 1. Graphical representation of the construction of each functional run. All stimuli types (graphic, suffering, and symbolic) were presented in block format. Each block consisted of the presentation of five pseudo-randomly selected stimuli of the appropriate type presented for 2 seconds each, and separated by 1 second of fixation. Block order was pseudo-randomized for each functional run.
229x238mm (227 x 227 DPI)

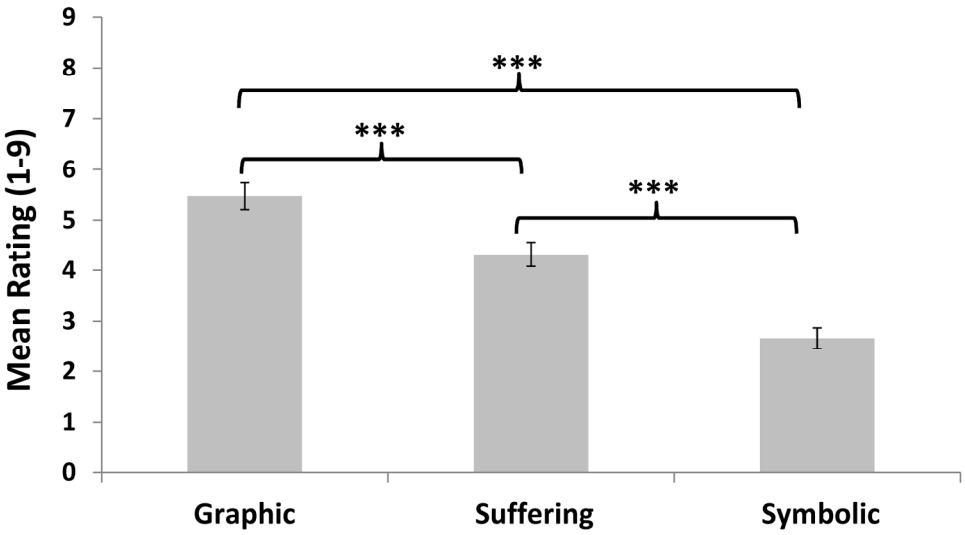


Fig. 2. Behavioral effectiveness ratings of HWLs. All participants rated all HWLs prior to fMRI scanning by responding to the question: "How much does this warning make you feel afraid?". *** = significant p < 0.001 (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM). 229x125mm (227 x 227 DPI)

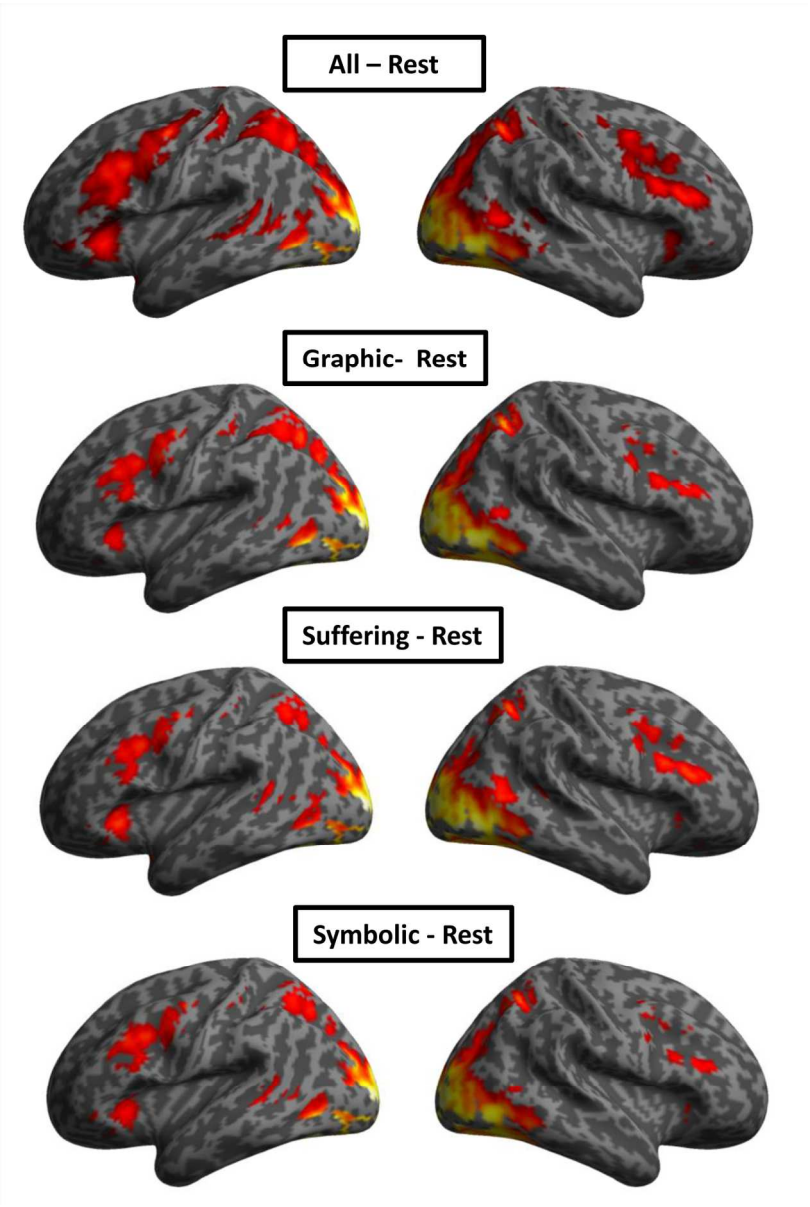


Fig. 3. Main effects of HWLs on BOLD signal (graphic, suffering, symbolic) on BOLD signal. All results are thresholded at p < 0.05 and corrected for family-wise error (FWE). Results are overlaid on a standard inflated brain (cortex_20484.surf.gii) for illustration purposes.
254x377mm (141 x 141 DPI)

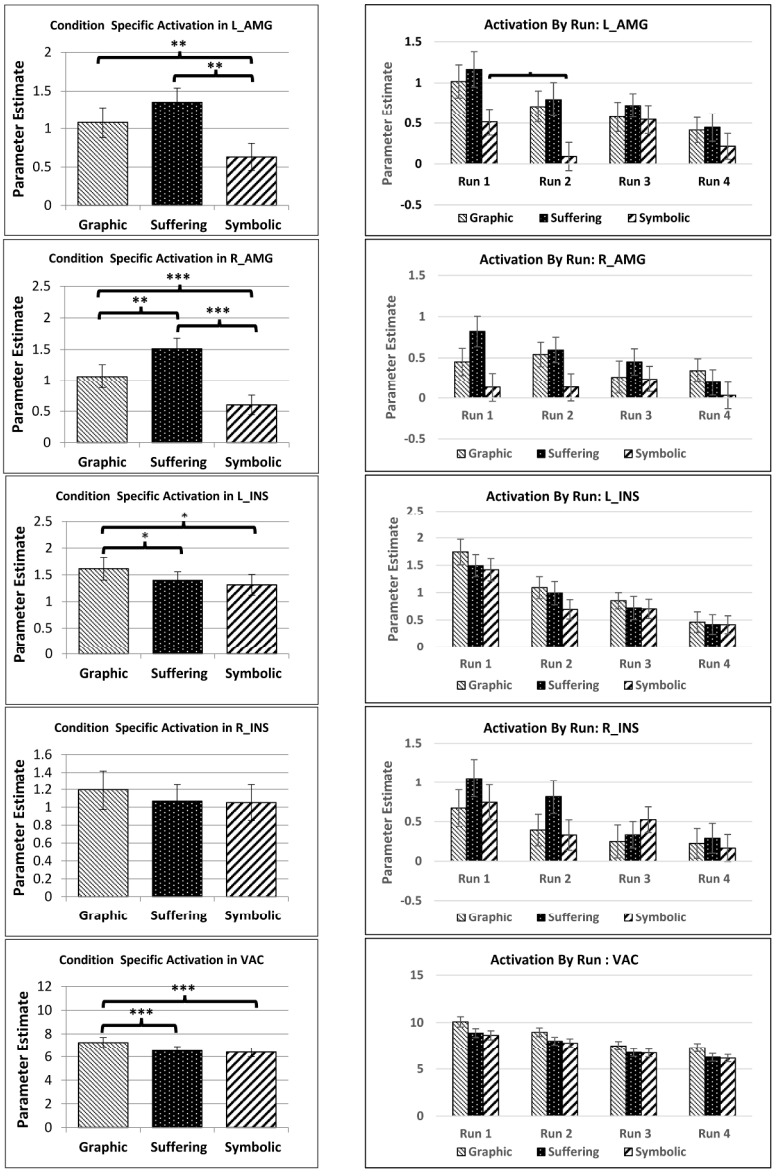


Fig. 4. (A) Results from ROI analyses. (B) Adaptation of BOLD signal in ROIs across four functional scanning runs. L_AMG = left amygdala {XYXmni = -26, -2, -17} , R_AMG = right amygdala {XYXmni = 23, 7, -17}, L_INS = left insula { XYXmni = -30, 30, 4}, R_INS = right insula { XYXmni = 28, 32, -8}, L_OCC = left occipital cortex{XYXmni = -26, -94, 4}, OCC = occipital cortex{XYXmni = -26, -94, 4; XYXmni = 24, -90, -6}, * = significant $p < 0.05$ (within subjects one-tailed t-test), ** = significant $p < 0.05$, *** = significant $p < 0.001$ (within subjects one-tailed t-test); Error bars represent standard error of the mean (SEM).
280x421mm (200 x 200 DPI)

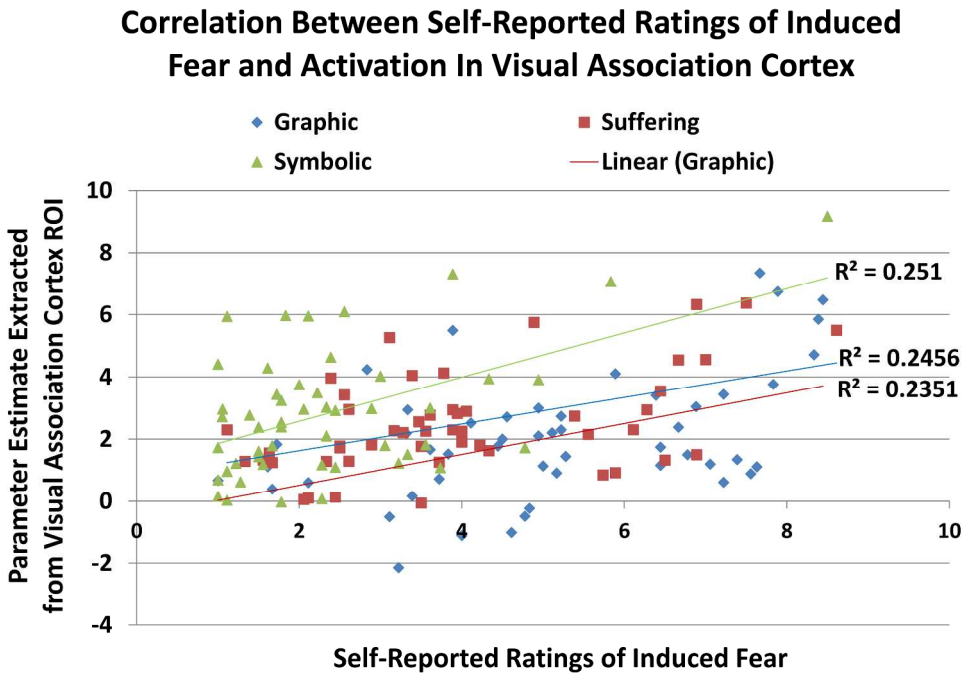


Fig. 5. Correlation between BOLD signal in the visual association cortex (BA 18) and participant self-reported ratings of different subtypes of HWL. The site of maximal correlation between the parameter estimates for the contrast (graphic-Rest) and self-reported ratings of graphic HWL stimuli was located at {XYXmni = -19,-92,20}. The site of maximal correlation between the parameter estimates for the contrast (suffering-Rest) and self-reported ratings of suffering HWL stimuli was located at {XYXmni = -20,-88,12}. The site of maximal correlation between parameter estimates for the contrast (symbolic-Rest) and self-reported ratings of symbolic HWL stimuli was located at {XYXmni = -14,-92,12}.
279x202mm (261 x 261 DPI)

Supplementary Table 1.

Demographic and Smoking Behavior Information		
Demographic Variables		n = 50, mean (SD) or %
sex	% female	48%
age	Mean	27.56
	Range	22
race	% White	74%
	% African American	24%
	% Other	2%
Education	High school or less	26%
	some college/tech school	55%
	college or more	18%
Income	low	63%
	middle	30%
	high	7%
Smoking/Consumer Behavior		
CO Level (ppm)		18.74 (10.57)
Cotinine Level (ng/mm)		207.48 (173.27)
Days Smoked (last 30 days)		28.32 (4.63)
Cigarettes (per day)		14.90 (10.09)
How worried smoking affects health?	not at all	0%
	a little worried	48%
	very worried	52%
Pay attention to HWLs	not at all	54%
	a little worried	40%
	somewhat	4%
	a lot	2%

Supp. Tbl. 1. Demographic and behavior information.

Supplementary Table 2.

Region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Graphic > Symbolic:					
*Lingual Gyrus	L	-16	-90	-8	11.98
*Primary Visual Cortex	R	22	-96	4	10.66
*Superior Parietal Lobule	L	-22	-70	40	6.07
*Superior Parietal Lobule	R	22	62	48	5.6
Inferior Parietal Lobule	L	-34	-38	44	4.69
Supramarginal Gyrus	R	60	-18	40	4.51
Amygdala	R	22	-4	-14	4.15
Precentral Gyrus	R	44	8	28	4.03
Inferior Parietal Lobule	L	-52	-28	36	3.96
Postcentral Gyrus	R	46	-30	44	3.76
Precentral Gyrus	L	-44	4	30	3.64
Amygdala	L	-20	-4	-12	3.6
Symbolic > Graphic:					
*Cuneus	R	4	-82	30	8.36
*Lingual Gyrus	R	10	-66	2	7.14
*Calcarine Gyrus	L	-8	-72	10	6.23
Supramarginal Gyrus	L	50	-34	22	4.63
Anterior Cingulate Gyrus	R	10	34	4	4.42
Middle Temporal Gyrus	R	54	-22	-6	4.40
Superior Temporal Gyrus	L	-52	-4	-12	4.27
IFG Pars Orbitalis	R	40	48	-4	3.74

L: left hemisphere; **R**: right hemisphere; **MNI :** Montreal Neurological Institute; **IFG** : Inferior frontal gyrus.
T-value: local maxima thresholded at $p < 0.001$, uncorrected, extent threshold $k = 10$
*values were significant after FWE correction, extent thresholding $k = 10$

Supp. Tbl. 2. Table of brain activations elicited by observation when comparing Graphic HWLs to Symbolic HWLs.

Supplementary Table 3.

Region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Suffering > Symbolic:					
*Fusiform Gyrus	R	42	-46	-18	8.99
*Post Middle Temporal Gyrus	R	54	-64	12	8.95
*Amygdala	R	20	-6	-10	7.85
*Precuneus	R	4	-58	38	7.03
*Hippocampus	L	-18	-8	-12	6.92
*Occipital Lobe	L	-46	-70	16	6.7
*IFG Pars Triangularis	R	42	18	24	5.89
*Hippocampus	R	18	-32	0	5.31
Ant. Middle Temporal Gyrus	R	58	0	-16	4.36
Orbital Frontal Gyrus	L	-2	56	-12	4.22
IFG Pars Triangularis	R	50	38	14	4.19
Cuneus	R	14	-95	14	3.96
Symbolic > Suffering:					
*Lingual Gyrus	L	-24	-58	-14	6.97
Lingual Gyrus	R	24	-58	-10	5.12
IFG Pars Triangularis	L	-38	42	10	4.78
Occipital Lobe	L	-30	-88	16	4.77
Anterior Cingulate	R	10	36	14	4.16
Superior Frontal Gyrus	R	22	50	10	3.70

L: left hemisphere; **R**: right hemisphere; **MNI :** Montreal Neurological Institute: **Ant.** : Anterior; **Post.** : Posterior; **IFG** : Inferior frontal gyrus.
T-value: local maxima thresholded at $p < 0.001$, uncorrected, extent threshold $k = 10$
*values were significant after FWE correction, extent thresholding $k = 10$

Supp. Tbl. 3. Table of brain activations elicited by observation when comparing Suffering HWLs to Symbolic HWLs.

Supplementary Table 4.

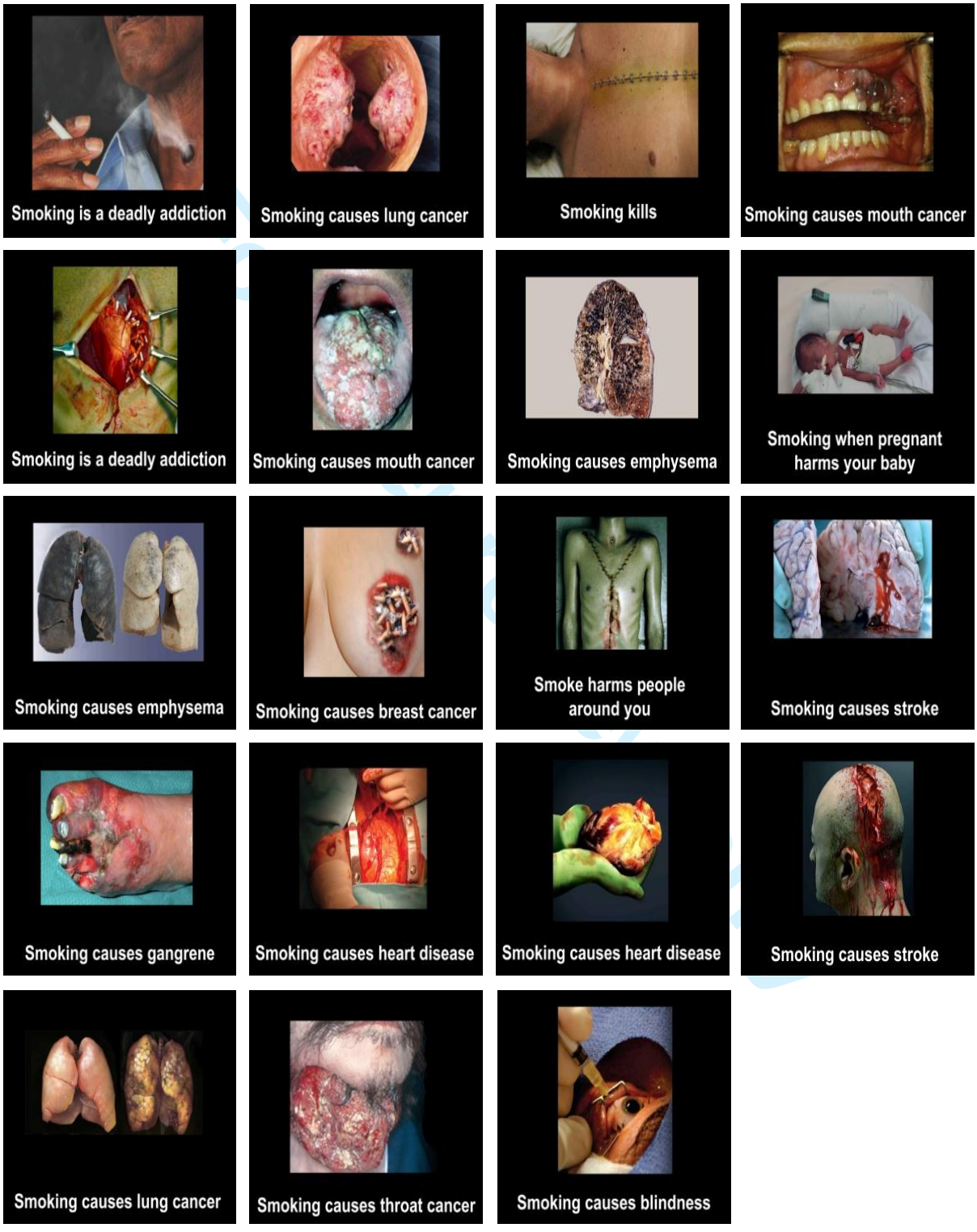
Region	L/R	local maxima peak coordinates (MNI)			T-value
		x	y	z	
Suffering > Graphic:					
*Post Middle Temporal Gyrus	R	50	-46	12	8.55
*Precuneus	R	4	-60	38	7.48
*Ant. Middle Temporal Gyrus	L	-54	-6	-12	6.87
*Ant. Middle Temporal Gyrus	R	56	-2	-16	6.42
*Post Middle Temporal Gyrus	L	-50	-50	12	6.39
*Orbital Frontal Gyrus	R	4	48	-12	6.20
*Lingual Gyrus	L	-12	-52	0	5.88
*Lingual Gyrus	R	12	-54	2	5.87
*Fusiform Gyrus	L	40	-45	-15	5.59
*Ant. Superior Temp. Gyrus	R	38	20	-28	5.43
*IFG Pars Triangularis	R	52	34	6	5.32
*Ant. Superior Temp. Gyrus	L	-46	10	-20	5.13
Hippocampus	R	28	-8	-14	4.77
Hippocampus	L	-20	-10	-14	4.24
Supplementary Motor Area	L	-2	-24	66	3.66
Graphic > Suffering:					
*Occipital Lobe	L	-30	-86	16	11.29
*Occipital Lobe	R	34	-82	12	10.67
*Fusiform Gyrus	L	-26	-56	-14	10.29
*Fusiform Gyrus	R	26	-56	-12	8.11
*Superior Parietal Lobe	R	26	-66	54	7.91
*Superior Parietal Lobe	L	-24	-74	36	7.24
*Inferior Temporal Gyrus	R	50	-56	-8	6.68
IFG Pars Opercularis	L	-46	2	30	5.03
Inferior Parietal Lobe	L	-40	-40	44	4.36
Middle Frontal Gyrus	L	-44	42	14	3.84
Supramarginal Gyrus	R	44	-32	44	3.77
Supramarginal Gyrus	R	50	-24	44	3.61

L: left hemisphere; **R**: right hemisphere; **MNI** : Montreal Neurological Institute; **Ant.** : Anterior; **Post.** : Posterior; **IFG** : Inferior frontal gyrus.
T-value: local maxima thresholded at $p < 0.001$, uncorrected, extent threshold $k = 10$
*values were significant after FWE correction, extent thresholding $k = 10$

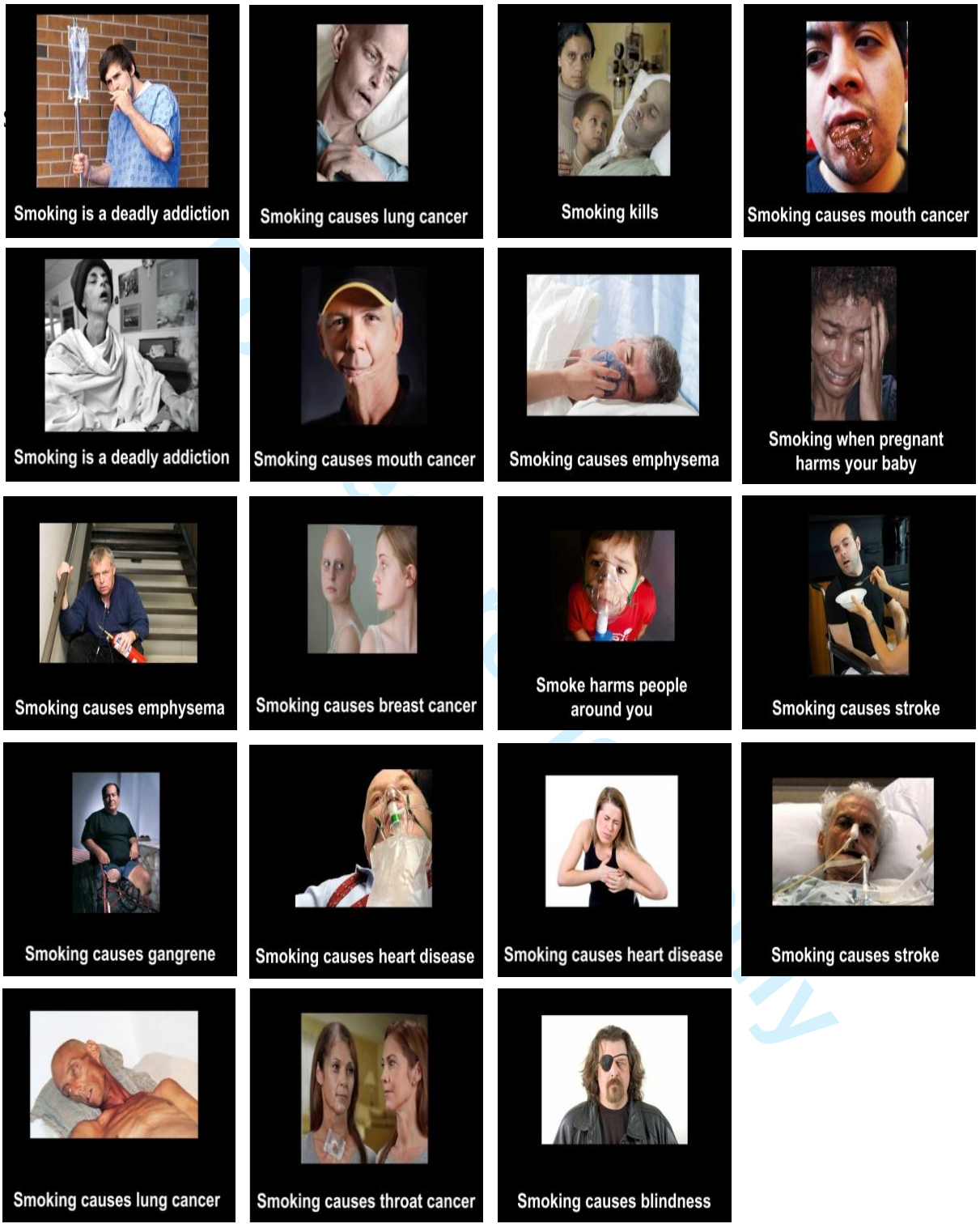
Supp. Tbl. 4. Table of brain activations elicited by observation when comparing Graphic HWLs to Suffering HWLs.

Supplementary Figure 1

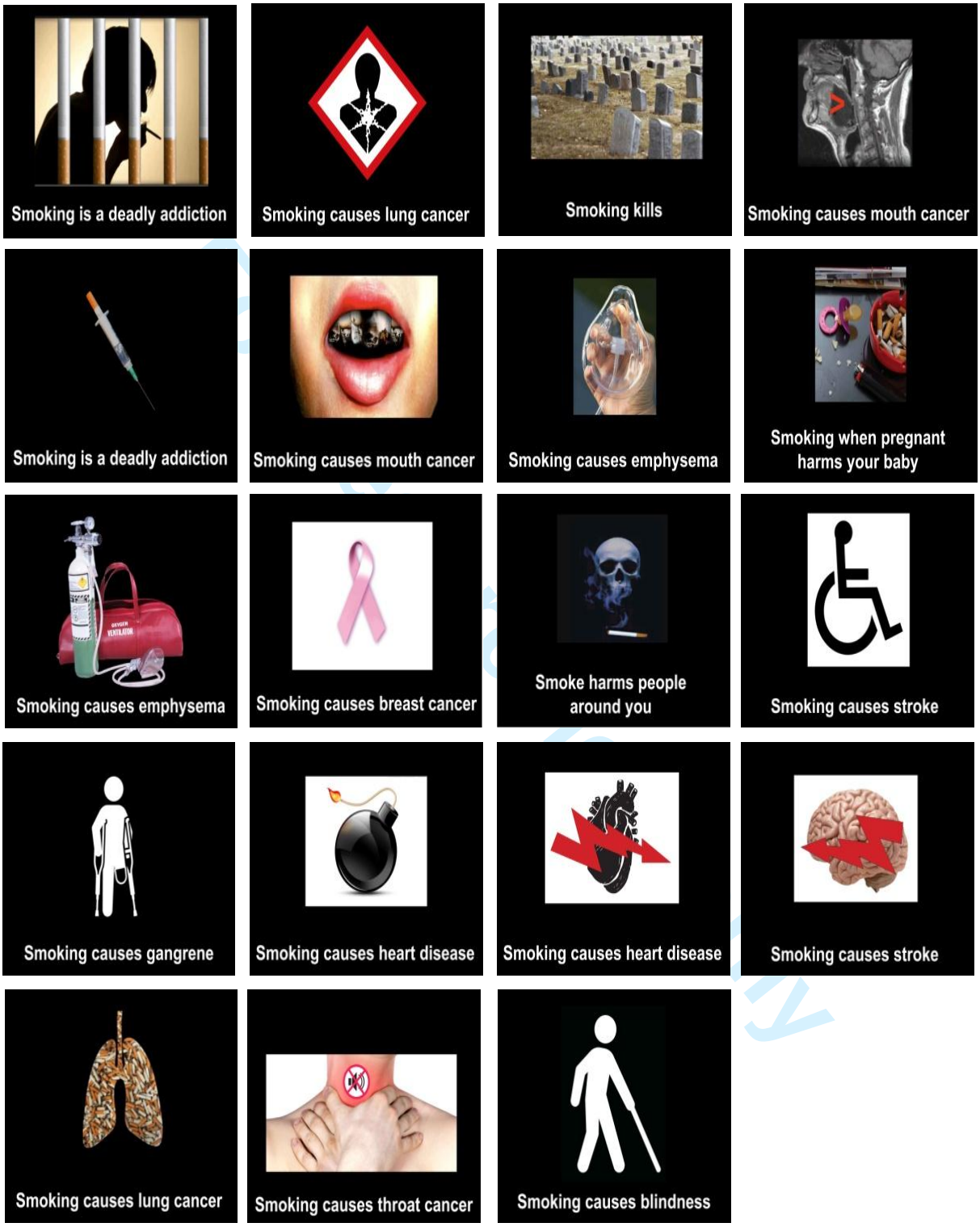
Graphic Images



Suffering Images



Symbolic Images



We confirm our compliance with the following STROBE statement recommendations for reporting cross-sectional studies.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a

		meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.