A behavioural and electrophysiological investigation of effects of visual congruence on olfactory sensitivity during habituation to prolonged odors

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Abstract

Congruent visual cues augment sensitivity to brief olfactory presentations and habituation of odor perception is modulated by central-cognitive processing including context. However, it is not known whether habituation to odors could interact with cross-modal congruent stimuli. The present research investigated the effect of visual congruence on odor detection sensitivity during continuous odor exposures. We utilised a multi method approach including subjective behavioural responses and reaction times (study 1), and electroencephalography (EEG, study 2).

Study 1: 25 participants received 2 minute presentations of moderate intensity floral odor delivered via olfactometer with congruent (flower) and incongruent (object) image presentations. Participants indicated odor perception after each image. Detection sensitivity and reaction times were analysed in epochs covering the period of habituation. **Study 2**: 25 new participants underwent EEG recordings during 145 s blocks of odor presentations with congruent or incongruent images. Participants passively observed images and intermittently rated perceived intensity of odor. Event-related potential analysis was utilised to evaluate brain processing related to odor-visual pairs across period of habituation.

Odor detection sensitivity and reaction times were improved by congruent visual cues. Results highlighted a diminishing influence of visual congruence on odor detection sensitivity as habituation occurred. ERP analysis revealed an effect of congruency on electrophysiological processing in the N400 component. This was only evident in early periods of odor exposure when perception was strong. For the first time, this demonstrates modulation of central processing of odor-visual pairs by habituation. Frontal negativity (N400) responses encode aspects of cross-modal congruence for odor-vision cross modal tasks.

Introduction

Integration of cross-modal sensory information in the brain is a dynamic, ongoing process that is subject to confounding top-down and bottom up influences which affect the individuals overall perception. Despite this, few studies investigate the interaction of olfactory-visual stimuli and, to our knowledge, none have considered whether typical confounding factors such as habituation to odor would affect this interaction. Whilst olfaction and vision operate via anatomically distinct brain pathways, both essentially serve the same function of object identification (Gottfried 2010). Research suggests a bi-directional relationship between vision and olfaction. Visual stimuli can facilitate odor detection (Gottfried and Dolan 2003) and identification (Dematte *et al.* 2009). Olfaction also influences fundamental aspects of visual processing, e.g., binocular rivalry studies (which present a different visual stimuli concurrently to each eye) show visual dominance occurring for the lateralised image matching the presence of a congruent, compared to incongruent, odor (Zhou *et al.* 2010). The precise mechanisms underlying the integration of olfactory and visual information in the brain are not fully understood, but may facilitate the effects of context and other top-down psychological influences (Robinson *et al.* 2015) or subjective experience (Amsellem *et al.* 2018).

Odor habituation describes the central-cognitive processes, s#uch as changes in brain and behavioral responsiveness or sensitivity to odor, which occur during prolonged periods of exposure (Dalton 2000). Neuroimaging research with Functional magnetic resonance imaging (fMRI) indicates that habituation is encoded in primary olfactory including piriform, entorhinal cortex and amygdala as well as higher order brain regions such as anterior insula and hippocampus (Poellinger *et al.* 2001). Understanding whether cross-modal cues would interact with the process of habituation has relevance for scientific understanding of the

interaction between these process, and also for commercial applications where long-lasting influence of fragrance is often desirable. Appropriate visual cues could affect olfactory processing during habituation by redirecting attentional resources. EEG studies have shown that focused attention increases olfactory event-related potentials (Geisler and Murphy 2000; Krauel *et al.* 1998; Masago *et al.* 2001; Pause *et al.* 1997), and fMRI studies report modulations of brain activation responses when attention is focused towards an odor (Plailly *et al.* 2008; Sabri *et al.* 2005; Veldhuizen and Small 2011; Zelano *et al.* 2005). Research from our group has also previously highlighted the influence of endogenous attention on the process of habituation (Fallon *et al.* 2018).

Traditionally, multisensory integration was thought to occur in higher order integrative brain processing regions, but more recent evidence suggests that at least some aspects are represented in primary sensory brain regions and directly affect perception (Liang *et al.* 2013; Meyer *et al.* 2011). Animal models using in vivo extracellular recordings from the olfactory tubercle have demonstrated interaction between olfactory and auditory processing in this primary olfactory cortex (Wesson and Wilson 2010). fMRI studies have demonstrated an interaction effect for activation in orbitofrontal cortex, inferior parietal lobule and posterior cingulate cortices during cross-modal odor-visual processing (Gottfried *et al.* 2004). However, to our knowledge no research exists to consider if effects of cross-modal interactions in primary or secondary olfactory cortices could be affected by olfactory habituation.

Previously, electroencephalographic (EEG) recordings were analysed using event-related potential analysis aligned to onset of images to study the effects of odors on visual processing (Bensafi *et al.* 2002; Castle *et al.* 2000; Grigor *et al.* 1999; Grigor 1995; Lorig *et al.* 1993; Lorig *et al.* 1995; Robinson *et al.* 2015). These studies generally utilised a variation

of an oddball paradigm, with a common-rare split of congruent and incongruent odor-visual pairs. The paradigm relies on the premise of olfactory priming, wherein the odor precludes the arrival of the visual stimuli and influences brain activity in a manner which leads to some quantifiable modulation of subsequent visual processing (Bensafi *et al.* 2002). We can index this modulation of visual processing using event-related potential (ERP) analysis of EEG data.

In previous research, the most common waveform modulated by odor-visual congruence is the N400, a negative deflection in frontal electrodes occurring from 250-500 ms after onset of visual stimuli which was previously proposed to encode the degree of congruence between an olfactory prime stimulus and the visual target (Bensafi *et al.* 2002). A recent review of N400 research concludes that it incorporates aspects of perception, attention, memory and semantics (Kutas and Federmeier 2011). The amplitude of this N400 wave was increased for incongruous and rare odor-visual pairs (Grigor *et al.* 1999; Grigor 1995), and modulation of the N400 wave has been demonstrated by studies using both pleasant (Sarfarazi *et al.* 1999) and unpleasant odor-visual pairs (Castle *et al.* 2000). Research from our lab previously identified modulation of the N400 component during affective face perception with hedonically congruent or incongruent odor priming (Cook *et al.* 2017). Together, this evidence suggests that odors may influence a late, semantic stage of visual processing as previously proposed (Grigor *et al.* 1999; Sarfarazi *et al.* 1999), although one recent study did not identify N400 differences and instead pointed towards an influence of odors on early (N1) visual processing (Robinson *et al.* 2015). It should be noted that few, if any, cross-modal EEG studies have focused on odor-detection outcomes.

All previous research of olfactory-visual interaction utilised short bursts of odor with long inter-stimulus intervals to prevent habituation. Therefore, it is not known whether the

effects of odor-visual congruence influence the process of olfactory habituation. Furthermore, the brain mechanisms which govern the interaction of odor-visual processing, and how these fluctuate during perceptual changes during prolonged odor exposure, are not known. In this research, we first investigated the influence of congruent visual cues on olfactory performance during a period of prolonged odor exposure to induce olfactory habituation. To determine whether habituation modulated central processing of olfactory–visual pairs, we analysed neural responses to congruent and incongruent visual stimuli across a period of prolonged odor using event-related potential analysis and distributed source localisation analysis of EEG. We hypothesised that congruent visual cues would lead to improved odor detection sensitivity, but that this improvement would reduce due to the process of olfactory habituation during a prolonged exposure. Furthermore, we expected that late-semantic components of processing for odor-visual pairs would be differentially affected by effects of cross-modal congruence, and that this effect would be modulated as habituation occurred.

Methods

Participants

For study 1, 25 participants (12 males) aged 24.2 ± 3.62 years (mean ± SD) were recruited. A separate cohort of 25 participants (13 males) aged 23.2 ± 3.99years (mean ± SD) took part in study 2. In both cases, participants were recruited through digital and campus advertisements at the University of Liverpool. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. The studies were approved by the University of Liverpool Research Ethics Committee. Participants aged between 18–35 years were considered for participation and volunteers taking regular medication, or those suffering from respiratory, neurological or olfactory disease or disorders (according to self-report) were excluded. Eligibility and sense of smell was assessed prior to the experiment

using the identification test from the 'Sniffing Sticks' odor test battery (Hummel *et al.* 1997). In this test, participants were asked to identify 12 odors from four visually presented options, and a minimum score of 9 correct probes was required for inclusion in either study. All volunteers were compensated for time and travel expenses.

Odor stimuli

For both studies the floral-green fragrance 'New Day' (Unilever Ltd) was utilised at 5% concentrations diluted in propylene glycol (1,2-Propanediol 95%, Sigma-Aldrich Co., USA). This concentration was found to be perceived as a moderate intensity following testing of a range of possible concentrations in psychophysical pre-studies (Unilever Ltd, unpublished).

Study 1

Procedure

Participants attended the EEG laboratory in the Department of Psychological Sciences at the University of Liverpool. Participants were seated 1 metre from a 19 inch computer monitor and a PneumoTrace II Piezo-electric transducer was fitted around the torso at the level of the epigastrium to record respiratory movements (ADInstruments Pty Ltd., Australia). The experiment consists of 10 blocks of prolonged (120 s) odor exposure at a flow rate of 2.2 litres/minute, there was a 1 minute rest period between blocks when participants were exposed to a constant flow of clean air, which was passed through pure propylene glycol solution with a matching flow rate. Each block consisted of 20 trials which lasted for 6 s each and consisted of a rest cross (1.5 s) followed by a picture presentation (0.5 s), blank screen (1 s) and a rating period (3s). Figure 1 shows the timeline of one experimental block. Each block contained 10 congruent pictures trials (flowers in a variety of arrangements on a

white background) and 10 incongruent stimuli (everyday objects on a white background). Each picture appeared twice in the experiment and the order of pictures was randomised in each block but conditions were alternated to maintain even spread of each congruent and incongruent trials throughout the period of odor exposure. During the rating period, participants were required to click either the left or right mouse button to indicate whether they detected any odor during the previous picture. Participants were informed that they may or may not smell an odor at any time during the experiment, that they should simply indicate of whether odor was present at that specific time. They were also instructed to give their response as quickly as possible. The lateralisation of the mouse button corresponding to detection was counterbalanced across participants.

The olfactometer utilised was custom-made, with 8 individual flow valves each benefitting from variable flow-rates and a carbon filtered air intake (OL-2, Dancer design Ltd., UK). Odors where delivered via fluorinated ethylene propylene tubing of 2 mm diameter extending 2 cm below the nostrils. During the experiment, the ambient air in the chamber was constantly cleansed of residual odor using a carbon filtered Blueair 203 Heppasilent Particle Filter system (Blueair AB, Sweden).

Odor detection and reaction time analysis

Bad trials (were neither option was selected during the 3 s response period) were removed for each participant, these represented less than 1% of total trials. Response data was divided into 5 time windows which each represented 24 seconds of odor presentations to evaluate whether the influence of congruent and incongruent stimuli differed across the period of odor exposure. Odor detection sensitivity for each picture condition and time window was calculated the percentage of trials in each block when participants correctly detected the presence of odor. Mean reaction time (RT) for accurate and inaccurate responses

was calculated in each subject for each time window and condition following removal of improbable response trials (RT > 1.5 s or RT < 0.2 s). Two-way within-subjects ANOVA analysis for odor detection and reaction time was performed in SPSS v.21 (SPSS Inc, Chicago, USA) to investigate effects of congruence (picture type; congruent-incongruent) and time (5 time periods of 24 seconds covering the 2 minute of exposure). Post-hoc t-tests were utilised to investigate significant interaction effects and a 95% confidence level was employed throughout.

Study 2

Procedure

The second study also occurred in the EEG laboratory in the Department of Psychological Sciences at the University of Liverpool. Olfactometer and respiratory monitoring setup were identical to study 1. EEG was recorded continuously using a 129-channel Geodesics EEG System (Phillips-Electrical Geodesics Inc., Eugene, Oregon, USA) with the sponge-based HydroCel Geodesic Sensor Net (HCGSN-128) with vertex reference. The sensor net was aligned with respect to three anatomical landmarks; two pre-auricular points and the nasion. The electrode-to-skin impedances were kept below 50 kW and the recording bandpass filter was 0.01−200 Hz. The sampling rate was 1000 Hz.

The second experimental paradigm consisted of 15 blocks, 10 utilised prolonged (145 s) odor exposure at a flow rate of 2.2 litres/minute and 5 blocks consisting of a continuous (145 s) flow of clean air at the same flow rate. Odorless blocks were interspersed evenly throughout the experiment with no two clean air blocks appearing consecutively (specifically in blocks 3, 5, 8, 10, 13). Order of blocks was consistent across participants. Again, a 1 minute clean air rest period was utilised between blocks. Each block consisted of 40 trials

which lasted for 3 s each consisting of a blank screen (2 s) followed by a picture presentation (1 s). The ratio of trials was skewed so that each block contained 30 object picture trials (expanded library from previous study) and 10 flower stimuli (same as previous). Each picture appeared three times in the total experiment. The order of pictures was pseudo-randomised in each block, with parameters to ensure that both conditions were dispersed equally across the block to allow for analysis including segmentation into time windows. At the beginning of each block, and every thirty seconds thereafter, a rating scale appeared on screen (5 s) with a visual analogue scale for participants to rate their perceived level of odor intensity at that moment using a mouse click. The scale anchors ranged from 'No odor' to 'Extremely Intense'. Figure 2 shows the timeline of one example experimental block.

ERP analysis

EEG data was pre-processed using BESA v.6.0 (MEGIS, Germany). Data was spatially transformed into reference-free data using common average reference method (Lehmann 1987) and downsampled to 256 Hz. Oculographic and electrocardiographic artefacts were removed using principal component analysis (Berg and Scherg 1994) in BESA v6.0 software. This is an interactive process where the user first manually identifies a prototypical eyeblink or electrocardiogram artefact complex in continuous data. This examplar complex is utilised in PCA to identify and remove all instances that match this pattern. No more than 2 artefact components (eye blink, electrocardiogram) were removed per participant and topographic maps of each individuals identified components were visually inspected to confirm typical topography before removal was performed. Data was visually inspected for the presence of movement or muscle artefacts, and epochs contaminated with artefacts were manually excluded. The mean number of trials remaining following artefact correction was 72.1 ± 6.52 (mean ± SD) for flower pictures in odor condition (72% of total

possible trials), 221.4 ± 18.8 for object pictures in odor condition (73%), 105.04 ± 12.14 (mean ± SD) for object pictures in clean air condition (70%), 35.96 ± 4.67 for flower pictures in clean air condition (72%).

ERPs associated with the onset of each type of picture in each odor condition were exported for the interval ranging from -200 ms to 1000 ms relative to stimulus onset (307 time points). This epoch was selected for ERP analysis as this period was found to adequately cover peaks in global field power and butterfly plots corresponding to the early, mid and long latency ERP components (Figure 5A). The baseline period was from -200 ms to 0 ms relative to the onset of the picture and EEG data was bandpass-filtered from 0.1 to 40 Hz. Finally, data was exported to Matlab v.8.10 (The Mathworks Inc, USA) for statistical analysis utilising EEGlab toolbox (Delorme and Makeig 2004).

To investigate the impact of odor habituation on processing of congruent or incongruent visual images, ERPs from each odor and picture-type condition were segmented into five 24 s time windows which covered the period of exposure. To identify electrodes and ERP components suitable for investigation, we employed a collapsed functional localiser method (Luck 2014) which utilises averaged data collapsed across one or more experimental manipulations to identify spatio-temporal clusters of interest. Grand-average ERPs representing all odor-picture pair conditions were initially divided into five levels corresponding to the 5 time windows covering the period of prolonged exposure. A non-parametric analysis was performed to investigate main effects of 5 levels of exposure time on brain processing of odor-visual pairs across all 129 electrodes and at every timepoint of the ERP. This analysis was performed using the Fieldtrip toolbox, implemented in EEGlab, and utilised 2000 permutations to counter the multiple comparisons required for investigation of spatio-temporal data (Maris and Oostenveld 2007). This analysis indicated a contiguous

cluster of frontal electrodes which demonstrated a significant effect of exposure time in the period 250-400 ms after picture onset (corresponding to frontal N400). Mean EEG voltages from this cluster and time period were exported for each participant in each odor, picture and time-window condition. N400 amplitudes were analysed using a 2×2 (odor×congruence) within-subjects ANOVA in SPSS v.21 (SPSS Inc, Chicago, USA) to investigate main effects and interactions of odor, picture type in each time window. A 95% confidence level was employed throughout.

Source reconstruction

Cortical sources of significant differences in ERPs were analysed using standardised Low Resolution Electromagnetic Analysis (sLORETA, (Pascual-Marqui 2002), implemented in LORETA v.200840-403 (*www.keyinst.unizh.ch/loreta*). sLORETA evaluates distributed electrical sources by smoothing the inverted images using a Laplacian smoothing operator to give cortical maps of electrical activity which show a good localisation accuracy (Greenblatt *et al.* 2005; Sekihara *et al.* 2005). Source maps were computed in a grid of 6239 voxels sized 5×5×5 mm^3, covering the entire cortical mantle. The sLORETA method was applied to localise the cortical sources contributing to the topographic configuration of ERP from any time window that demonstrated significant effects identified in scalp level analyses. Grand average sLORETA maps were generated representing the strongest cortical sources associated with all conditions using 5000 randomisations and an arbitrary T threshold (T > 15) was implemented to restrict maps to distinct cortical structures indicative of strongest cortical sources of scalp ERPs. Then, sLORETA values from each of these sources and for each condition were exported using 10mm diameter spherical region of interest (ROI) centred on the peak value of each cluster. The extracted values for each ROI were utilised in a 2×2

(odor×picture type) within subjects ANOVA to consider effects of congruence on source level activations in ERP components which demonstrated significance in scalp data.

Results

Study 1

Subjective responses for perceived odor presence were recorded after each picture presentation and mean odor detection sensitivity (%) was calculated for each subject in each of 5 (24 s) time windows covering the period of odor exposure. A 2×5(picture type × time) within subjects ANOVA revealed a main effect of picture type ($F(1,24) = 17.33$, $P < 0.001$), with a greater proportion of accurate odor detections when flower pictures where presented. There was also a significant main effect of time ($F(4,96) = 27.96$, $P < 0.001$) with greater odor detection sensitivity evident in early time windows of the odor exposure. The standard interaction effect was not significant ($F(4,96) = 2.38$, $P = 0.57$), but the cubic interaction effect was highly significant ($F(1,24) = 8.51$, $P = 0.008$). This cubic polynomial represents an exponential model of change in odor detection sensitivity in congruent compared to incongruent conditions from early to later time windows of the odor exposure. Post-Hoc t-tests indicate that congruent images lead to more accurate odor detection which is strongest in the first time window ($t(24) = 6.44$, $P < 0.001$), an effect that continues throughout the period of odor exposure until the difference is no longer significant in the final time window when odor intensity is lowest ($t(24) = 1.1$, $P = 0.28$). Figure 3A shows the mean sensitivity rate of detection for each time window.

Reaction times for correct odor detections following each type of picture presentation were exported and segmented into identical time windows. A 2×5(picture type × time)

ANOVA for RT's revealed a significant effect of picture type ($F(1,24) = 12.07$, $P = 0.002$) demonstrating shorter reaction times required to correctly identify the presence of odor in the congruent, relative to incongruent condition. However, the main effect of time was not significant ($F(4,92) = 2.20$, $P = 0.75$), nor the interaction ($F(4,92) = 0.24$, $P = 0.92$). Figure 3B shows the mean reaction time for accurate responses in each time window of the odor exposure.

Study 2

Subjective intensity ratings

Subjective ratings of perceived odor intensity were recorded in 30 s intervals throughout each block. A 2×5(odor×time) within subjects ANOVA revealed a main effect of odor, with greater intensity ratings reported across all time points when odor was present ($F(1,24) = 169.47$, $P < 0.001$). There was also a main effect of time, with higher odor ratings given at earlier time points in the exposure ($F(1,24) = 30.80$, $P < 0.001$). The interaction effect was also significant, indicating a difference in the effect of odor presence across the different time periods. ($F(1,24) = 18.63$, $P < 0.001$). Post Hoc paired samples t-tests reveal a significant difference in perceived odor intensity between odor and clean air blocks at every time point as would be expected given the nature of the comparison. However, data indicate that the difference between odor conditions was greatest at the start of the presentations, and decreased in a linear fashion indicative of habituation as the exposure prolonged (Figure 4).

ERP analysis

To investigate effects of odor-visual congruence across the period of exposure, EEG amplitudes from a cluster of fronto-central electrodes in the period 250-400 ms after picture

onset (which demonstrated a main effect of prolonged odor presentation time in the localiser) were investigated. Visual inspection of ERPs and topographic maps for this period displayed a consistent negative potential component over frontal-central regions corresponding to N400. Figure 5 shows the butterfly plot of the grand average ERP data encompassing both odor and picture congruence conditions. N400 amplitudes corresponding to this time period and electrode cluster were exported for each individual participant for analysis utilising 2×2 within subjects ANOVAs (odor×congruence) for each of the five time windows.

A 2×2 (odor×picture type) within subjects ANOVA for the first time window (when odor perception was strongest) revealed a main effect of odor, with stronger frontal negativity from 250-400 ms in odor blocks relative to clean air blocks ($F(1,24) = 6.34$, $P = 0.019$). There was no significant effect of picture type for trials from the first (early exposure) time window, but the interaction was significant ($F(1,24) = 7.37$, $P = 0.012$) indicating a difference in the effect congruent, relative to incongruent, pictures depending on the presence of odor. Paired samples t-tests indicated greater N400 negativity in odor trials with congruent images ($t(24) = -2.94$, $P = 0.007$), which was not evident in clean air trials ($t(24) = -1.36$, $P = 0.19$). Figure 5 shows the average ERP curves from significant electrodes and bar charts illustrating mean amplitudes for each group and condition in the first time window, scalp isopotential maps of ERP components for each group and picture type are shown averaged across the N400 time window. ANOVAs for the subsequent time windows covering the remainder of the prolonged odor exposure revealed no significant main effects of odor/picture type or significant interactions.

Distributed source analysis

The sLORETA output for peak source generators of topographic ERPs in the N400 time window (250-400 ms) are illustrated in figure 6. The activations represent grand average

of activity for all conditions in the first time window of odor exposures. Univariate analysis of sLORETA maps revealed 12 distinct clusters of activation. These regions included, bilateral orbitofrontal cortices; right inferior frontal gyrus; bilateral insula cortices; anterior, mid and posterior cingulate cortex, bilateral parahippocampal gyri; bilateral lingual gyri and right parietal cortex. These regions represent the peak sources of activation during the N400 component period for all conditions in the first period of odor and clean air blocks. We exported sLORETA values from each of these sources and for each condition using a spherical (10mm diameter) region of interest (ROI) centred on the peak value of each cluster. The values for each ROI were utilised in a 2×2 (odor×picture type) within subjects ANOVA but no regions exhibited any significant interaction effect which survived correction for multiple comparisons.

Discussion

The findings of study 1 revealed an effect of odor-visual congruence for improved odor detection sensitivity which persisted throughout the period of exposure. However, odor detection sensitivity diminished over time and the cubic interaction between odor and picture type points to a fading influence of odor-visual congruence as habituation occurs. This supports our first hypothesis. Reaction time data for accurate odor detections in the congruent condition were shorter throughout the entire exposure, pointing to an influence of odor-visual congruence on central processing, but there was no evidence of any difference as the exposure progressed. This demonstrates that congruent visual cues did not significantly reduce the degree or scale of habituation, although they do improve detection sensitivity consistently across the whole exposure period. Study 2 expanded on the previous study to elicit understanding of how central processing of odor-visual perception may be affected by

the influence of congruence throughout habituation to prolonged exposure. ERPs relating to onset of picture presentation in the presence or absence of odor point to changes in electrophysiological processing of images over the period of exposure. A significant interaction effect (i.e., an influence of odor-visual congruency) was evident in the N400 frontal negativity during the early period of odor exposure when perception was at its strongest level. During this early period when odor perception was strongest, congruent odor-visual pairs elicited the strongest N400 negativity. Source analysis of the N400 component from trials in the early time window revealed a complex array of active sources relating to the scalp data.

Our behavioural findings revealed that congruent odor-visual pairs resulted in a shorter response time and improved sensitivity which corresponds with previous research (Gottfried and Dolan 2003). However, we have expanded on this by demonstrating that, although congruence affects performance throughout the entire prolonged exposure period, habituation diminishes odor perception sensitivity and the influence of visual congruence. Previous studies reported enhanced frontal negativity with odor, relative to clean air, in an odor-visual pair paradigm (Lorig *et al.* 1993; Lorig *et al.* 1995), which accords with our own findings and which may suggest that frontal negativity is indicative of the influence of odor primes on subsequent visual processing. However, several previous studies have indicated that incongruent odor-visual pairs may be accompanied by enhanced N400 negativity (Castle *et al.* 2000; Grigor *et al.* 1999; Grigor 1995), which diverges from the congruence effect seen in the present study. However, these studies all utilised a minority (25%) of rare *incongruent* trials (opposite to the balance in the present study) and also requested explicit response to categorise stimuli as congruent or incongruent (not odor detection). In the present study, enhanced N400 could stem from the fact that *congruent* odor-picture pairs are the rare event in the current paradigm (at a frequency of 25%), and therefore represent an expectation

violation that could result in enhanced frontal negativity. Our findings suggest that the N400 may encode aspects of salience of the stimuli, which can be boosted by either congruence or incongruence depending on rarity or context. This interpretation would be in agreement with the opinion that the N400 response may pertain to stimuli that violate a previously established context (Pratarelli 1994). An electrophysiological review also concluded that N400 represents a signature of complex processing encompassing aspects of perception, attention, memory, and semantics which combine to influence the manner in which we infuse our environment with meaning (Kutas and Federmeier 2011).

In light of the literature, it would be overly simplistic to infer that the N400 component in response to odor-visual pairs relates directly and solely to congruence, and this may explain why some previous crossmodal studies failed to elicit N400 differences (Bensafi *et al.* 2002; Robinson *et al.* 2015). Instead, we can view our findings, and previous research, as indicative that the N400 represents a valid research target for aspects of odor-visual processing which impact on interpretation of our environment. However, further research is required to fully elucidate the mechanisms by which this modulates perception. For example, in the present study we are limited by the fact that congruence effects cannot be dissociated from effects of rarity of stimuli. Furthermore, different odors offer different profiles of habituation (Sinding *et al.* 2017), which may also play a role in subsequent interaction with visual cues. In future, it is possible that this experimental paradigm can be expanded to better facilitate the N400 as a research target, e.g., by using a wider range of odor-visual congruence pairings to better elucidate congruence (Sarfarazi *et al.* 1999), or by refining the paradigm to incorporate participant feedback in the form of categorising the congruency of each pairing which was shown to improve N400 modulation in relation to context in previous studies.

One previous study utilised odor-visual pairs with an exposure time of 1 minute, but the researchers did not analyse the effect of time on central processing of odor-visual pairs (Sarfarazi *et al.* 1999). Therefore, our finding of N400 effects diminishing as odor exposure progresses gives the first indication that this component could be modulated by ongoing habituation to odor. Source localisation of the significant congruence effect in N400 points to a complex array of cortical sources, but perhaps of most relevance is the inclusion of bilateral orbitofrontal, insula and parahippocampal sources, and cingulate sources during the N400 processing time period. Occipital sources in bilateral lingual gyri are most likely related to concurrent visual processing. Parahippocampal regions and orbitofrontal cortex were previously highlighted as regions with importance for the integration of odor-visual input using fMRI (Gottfried and Dolan 2003), and positron emission tomography revealed an integrative role for insula activation which was only present in cross-modal olfactory-gustatory processing (Small *et al.* 1997). Previously, the conditioning of congruent odor-visual stimuli was shown to evoke olfactory-like activation in orbitofrontal, insula, hippocampal and cingulate cortices for subsequent visual stimuli (Karunanayaka *et al.* 2015). Therefore, these regions are likely to be important for the interaction of odor-visual processing which contributes to a holistic percept in the human brain.

To conclude, together our studies demonstrate an interaction between congruence of cross-modal odor-visual pairs and the ongoing process of habituation to odor. This highlights the existence of a relationship between of top-down psychological factors and the habituation process. Our previous research highlighted the influence of attention on habituation (Fallon *et al.* 2018), but the present findings begin to shed light on the impact of habituation on central-cognitive cross-modal processing. The findings support the bidirectional relationship between odor-visual processing; congruent visual cues influence behavioural measures of odor perception, and odor also effects electrophysiological processing of visual cues. For the first

time we show evidence of a shift in the relationship as odors prolong and habituation occurs. Our findings also indicate support for the N400 component as a potential marker of the influence of context and congruence during odor habituation with cross-modal visual stimuli.

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Figure Legends

Figure 1. Flowchart of an example block from Study 1. Each odor block contained 20 (6 s) trials comprising a rest cross (1.5 s), picture presentation (0.5 s), blank screen (1 s) and a response period (3s).

Figure 2. Flowchart of an example block from Study 2. Each block of odor or clean air block contained 40 trials which lasted for 3 s consisting of blank screen (2 s) followed by a picture presentation (1 s) with 30 incongruent and 10 congruent trials. Participants rated odor intensity at the beginning of each block and every 30 s thereafter.

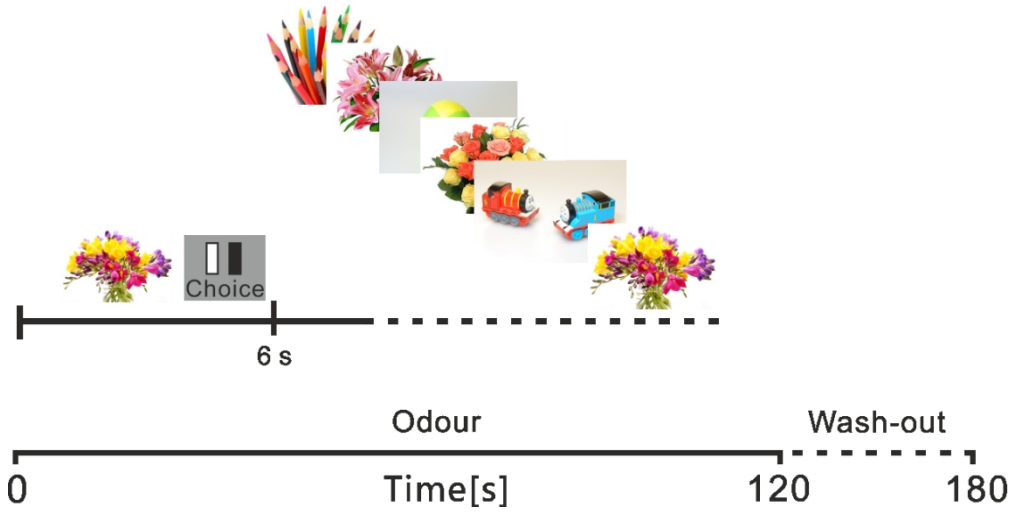
Figure 3A. Mean odor detection sensitivity (%) throughout the period of odor exposure, and **B**, mean reaction time (s) for correct responses to odor detection for congruent (blue) and incongruent (red) odor-visual pairs. Error bars illustrate 95% confidence intervals.

Figure 4. Mean subjective ratings of odor intensity throughout the period of exposure for clean air (red) and odor (blue) blocks. Error bars illustrate 95% confidence intervals.

Figure 5A. The butterfly plot of grand averaged data from all odor and picture conditions representing the ERP associated with onset of picture stimuli from all electrodes. The period of N400 negativity established from previous studies is highlighted. **B** Mean event-related potential for each picture type and odor condition in the early period of odor/clean presentations. The ERPs represent the average data from the cluster of electrodes identified by omnibus analysis (white circles panel D), the grey rectangle indicates the period demonstrating a significant interaction between odor condition and picture type. Red = Odor condition with incongruent object pictures; blue = odor and congruent flower pictures; Green = clean air with object pictures; black = clean air condition with flower pictures. **C** Bar chart illustrating the mean amplitude and standard error bars for the N400 component (250-400 ms, grey rectangle,

Panel B) from select electrodes. **D** Scalp isopotential maps demonstrating the topography of the ERP for each condition during the period 250-400 ms after picture onset.

Figure 6. Axial montage illustrating the peaks of source activation throughout the whole brain identified by univariate analyses (T>15) of grand average data in the early time window (which demonstrated an interaction between odor and picture type in scalp analyses).



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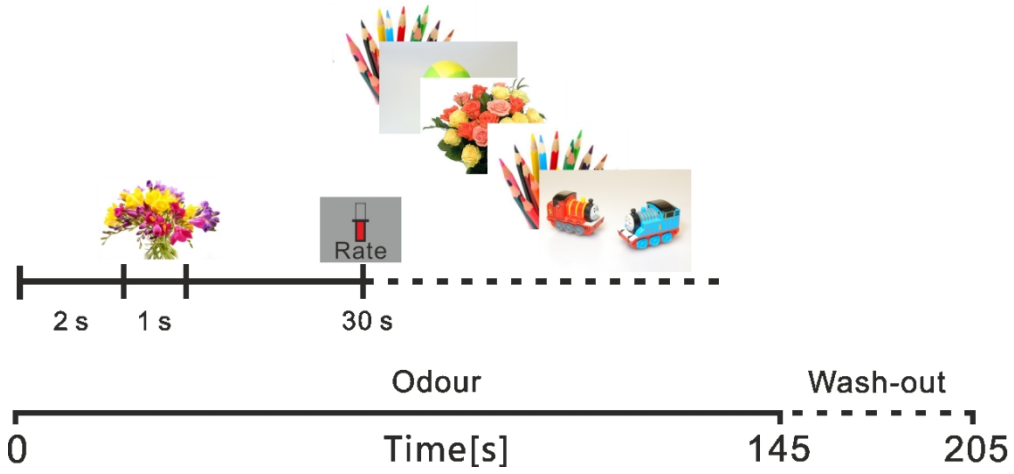
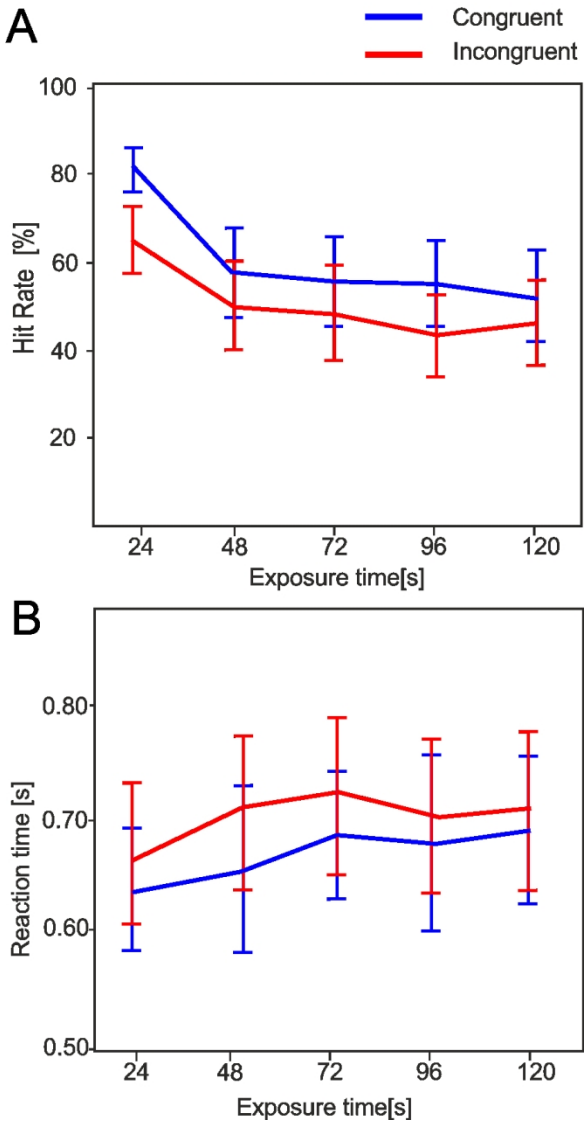


Figure 2. Flowchart of an example block from Study 2. Each block of odor or clean air block contained 40 trials which lasted for 3 s consisting of blank screen (2 s) followed by a picture presentation (1 s) with 30 incongruent and 10 congruent trials. Participants rated odor intensity at the beginning of each block and every 30 s thereafter.



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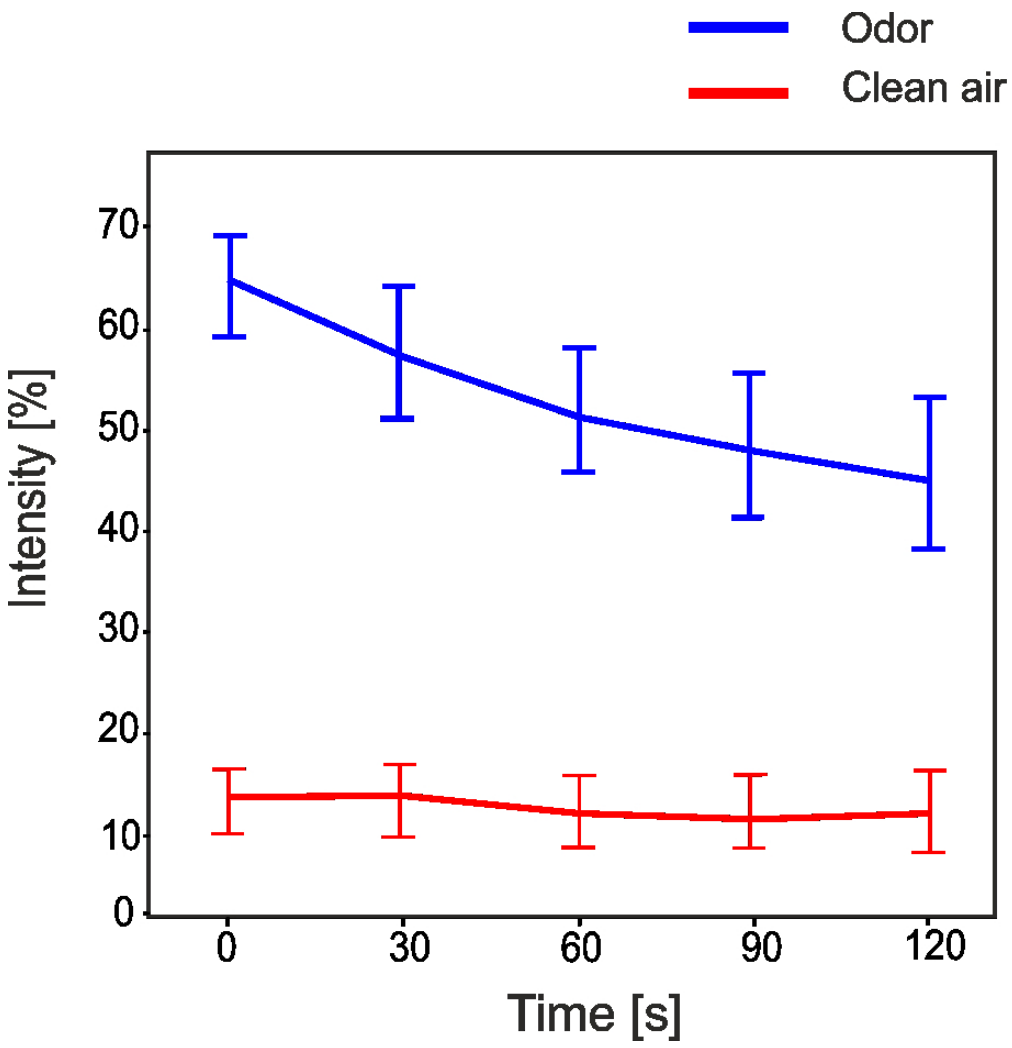

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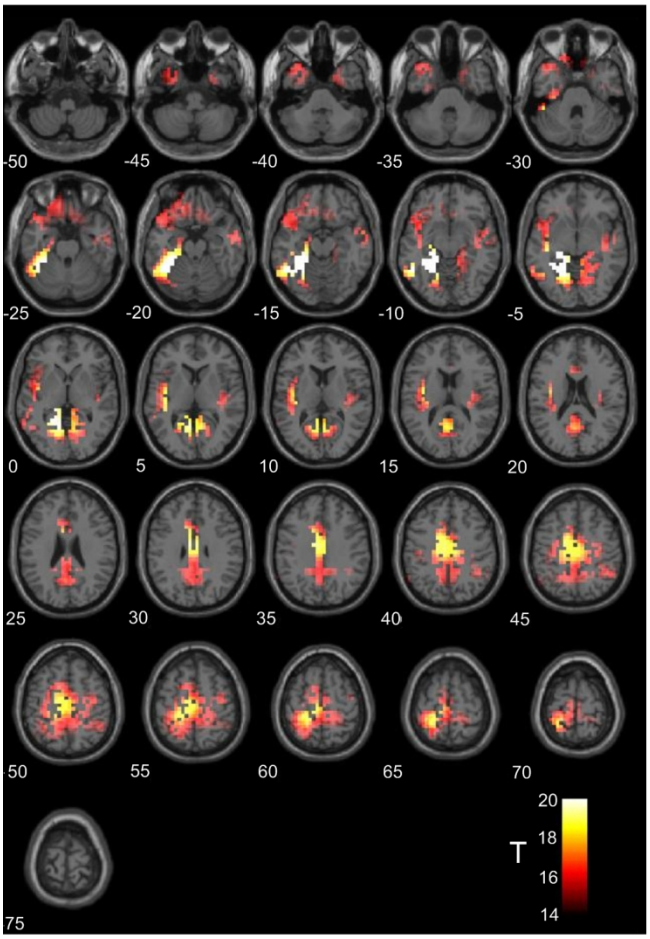

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