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Measuring the postauricular reflex as an indicator of appetitive processing

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Abstract

The postauricular reflex is a muscular microreflex behind the ear that is elicited by brief and abrupt sounds. Although it is vestigial in humans, it has been shown to have a potentiated response to the eliciting sound during the presentation of stimuli usually considered as pleasant relative to neutral or unpleasant stimuli across different sensory modalities; this response being even more prominent for appetitive stimuli, such as food and erotic images. This reflex can thus serve as a psychophysiological indicator of appetitive processing and could be a valuable tool for food science. Here, we describe a protocol that can be used to measure and analyze the postauricular reflex in humans. Our goal is to provide information on the materials and methods needed to record the postauricular reflex and to illustrate the preprocessing steps and quantification strategies that can be implemented to score this reflex.

Keywords: postauricular reflex, appetitive processing, emotion, positive affect, psychophysiology, electromyography

1. Introduction

The postauricular reflex is a muscular microreflex occurring behind the ear; it pulls the ear upward and backward ***(1)*** in response to brief and abrupt sounds, such as loud noises or noise clicks. It was first reported in humans as an electrical potential over the postauricular muscle evoked by click sounds ***(2)***. The postauricular reflex is generally imperceptible in humans (i.e., it does not produce visible motion in the pinna) and measured using surface electromyography (EMG) by placing electrodes over the postauricular muscle (see Fig. 1) behind the ear ***(3)***. This reflex has a typical response latency of 9-11 ms following the onset of an acoustic probe, showing a rapid response that is even faster than other reflexes similarly triggered by sudden and intense sounds such as the startle eyeblink reflex (latency of 45-50 ms) ***(4)*** (see **Note 1**). The neural circuitry of the postauricular reflex involves a multisynaptic pathway from the ventral cochlear nucleus to the trapezoid body in the caudal pons to the medial subdivision of the facial motor nucleus, which in turn activates the postauricular muscle ***(5,6)*** (see Fig. 2). Importantly, even though the postauricular reflex is vestigial in humans, it is still modulated by multiple attentional and affective factors and has the potential to provide information about a variety of psychological processes ***(6)***.

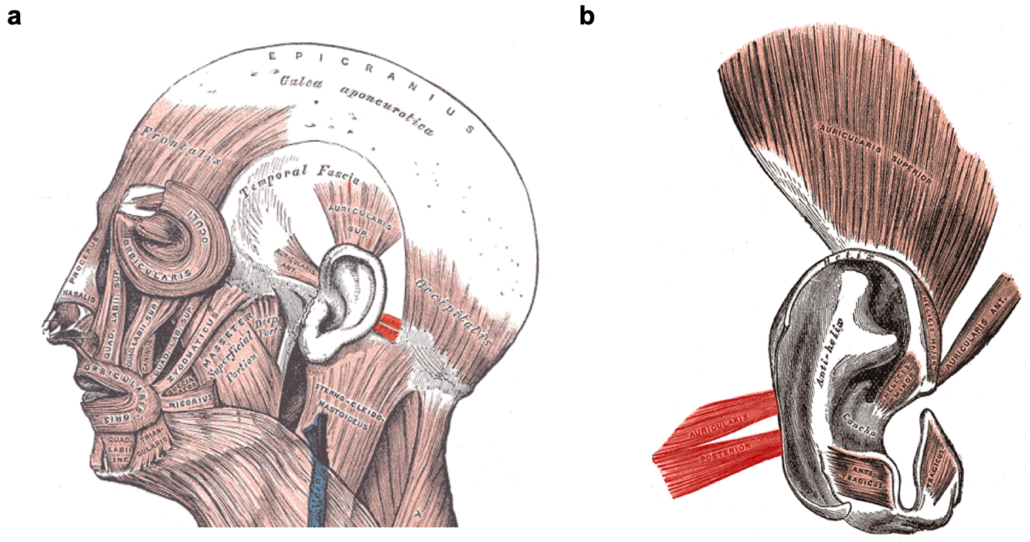

Fig. 1 Illustration of the postauricular muscle. Location of the postauricular muscle (highlighted in red) in relation to (a) the facial muscles and (b) the auricular muscles. Modified from ref. ***(36)***. In the public domain.

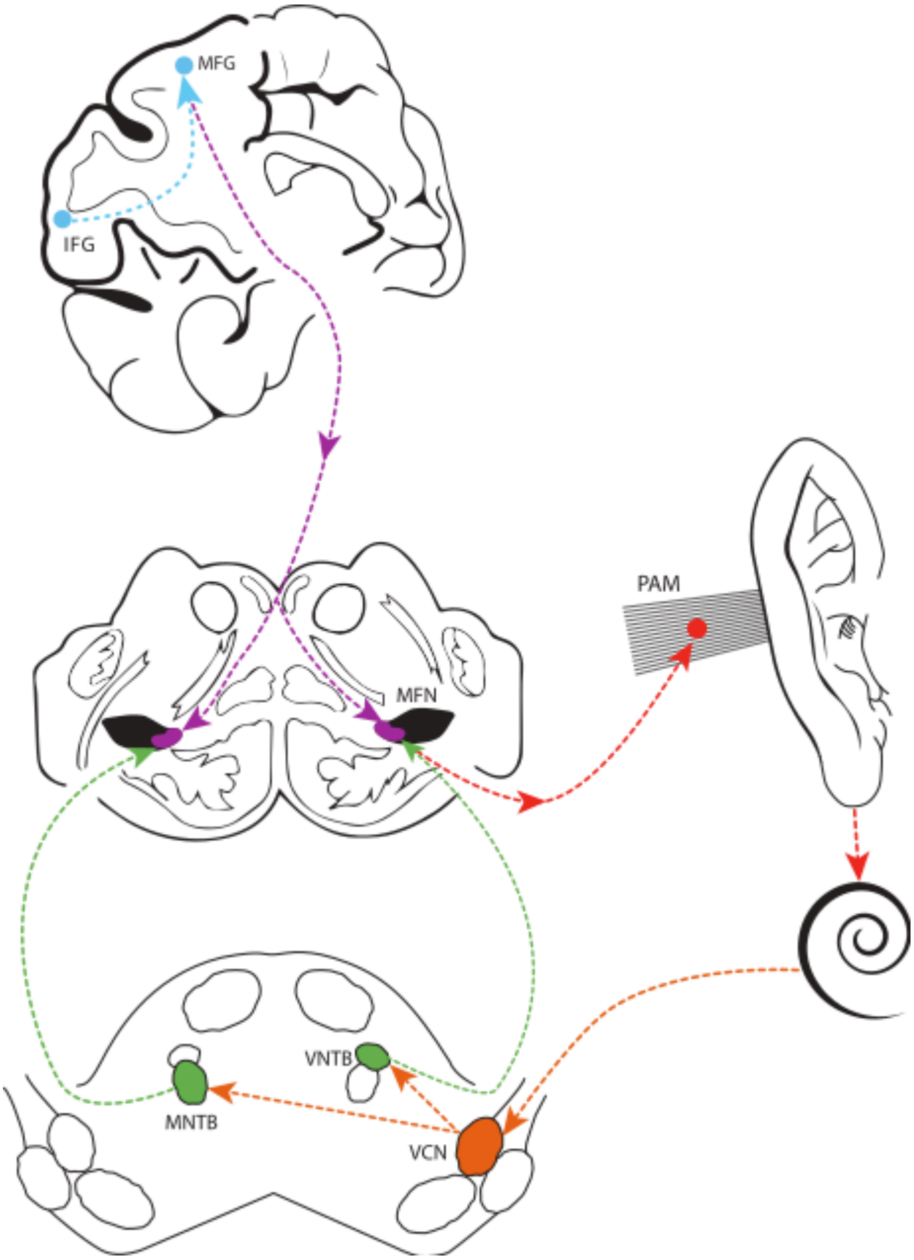

Fig. 2 Posterior view of a model of the postauricular reflex neural circuitry. The lower half (orange, green, and red arrows) shows the neural circuitry generating the postauricular reflex. The upper half (blue and purple arrows) depicts the putative neural activity that potentiates the postauricular reflex amplitude during pleasant versus neutral stimuli (see ref. ***6***). The spiral shape on the figure represents the cochlea. VCN = ventral cochlear nucleus, VNTB = ventral nucleus of the trapezoid body, MNTB = medial nucleus of the trapezoid body, MFN = medial facial nucleus, PAM = postauricular muscle; IFG = inferior frontal gyrus (left), MFG = middle frontal gyrus (left). Figure by Natosha D. Benning and Stephen D. Benning (2017); available at https://doi.org/10.6084/m9.figshare.4233098 under a CC-BY4.0 license and adapted from ref. ***(37)***.

For instance, studies in humans have shown that attention can modulate the amplitude of the postauricular reflex ***(4-7)***. It has been reported that the postauricular reflex is larger behind the attended ear in comparison to the unattended ear in an auditory detection task ***(4)***. The postauricular reflex also exhibits the attentional and sensory phenomenon of prepulse inhibition ***(4,7)***--in which the reflex is inhibited by the occurrence of a weaker stimulus (prepulse) preceding the reflex-eliciting stimulus (pulse)--at short lead intervals (i.e., time between the prepulse and the pulse; e.g., 100 ms). Longer lead intervals (e.g., 300 ms) conversely elicit prepulse facilitation of the postauricular reflex ***(7,8)***--which consists in the opposite reaction wherein the reflex is potentiated following the presentation of a less intense prepulse stimulus. Attention to the prepulse stimulus, however, does not enhance the prepulse inhibition of the postauricular reflex, thus differentiating it from the startle eyeblink reflex ***(4)*** and possibly reflecting its simpler neural circuitry ***(9)***. Moreover, continued attention to visual or auditory foreground stimuli inhibits the postauricular reflex, the more complex the foreground stimulus the greater the postauricular reflex inhibition ***(6)***. Indeed, evidence shows that the sound-evoked postauricular reflex amplitude is generally smaller during simultaneous picture or sound processing than during intertrial intervals containing only a fixation cross ***(10-12)***.

In addition to its attentional modulation properties, a key characteristic of the postauricular reflex is its sensitivity to affective modulation. Research has shown that the postauricular reflex in response to the eliciting sound is specifically potentiated during the presentation of pleasant stimuli compared to neutral or unpleasant stimuli across different sensory modalities ***(8,10-18)***. More precisely, in the visual domain, the postauricular reflex magnitude is the largest when viewing appetitive images, such as images of food or erotic scenes, and larger than when viewing other types of positive images that do not directly relate to appetitive (approach) motivation, such as images of adventure or pleasant nature scenes ***(6,18)***. This indicates that the postauricular reflex can provide a psychophysiological index of appetitive processing in humans ***(6,10,11,18)***. Importantly, a growing body of evidence further suggests that the postauricular reflex is not only modulated while experiencing the appetitive or pleasant stimuli themselves ***(10,12,13,18)***, but also in response to visual cues that predict the occurrence of a rewarding outcome (i.e., in anticipation of the reward) such as a pleasant odor ***(11)*** or juice ***(19)***. Of note, the postauricular reflex potentiation to the cues extinguished when the reward was no longer delivered ***(11,19)***, thus highlighting that the postauricular reflex is sensitive to appetitive contingencies. These findings suggest that the postauricular reflex possibly tracks the current reward value of the stimulus, which likely reflects the interplay of multiple reward components and not exclusively positive valence ***(20,21)***. In that sense, the postauricular reflex appears to be a relevant psychophysiological measure in the study of affective processes related to food stimuli and could be a valuable tool for food science. For instance, specific eating disorders such as binge eating and restrictive eating have been shown to increase and decrease, respectively, the postauricular reflex reactivity in response to food images (***17,22***), highlighting that the postauricular reflex can be used to assess appetitive motivation to food. More generally, the postauricular reflex could offer an indirect psychophysiological index to characterize the appetitive properties of food stimuli without requiring relying on (or in addition to) subjective reports.

In this vein, we report here a protocol that can be used to measure the postauricular reflex. This protocol is based on our work characterizing this reflex as an indicator of appetitive olfactory conditioning in humans ***(11)***, wherein the sound-elicited postauricular reflex is potentiated during the presentation of a stimulus predicting the delivery of a pleasant odor compared to a stimulus paired with odorless air. We first detail the materials and procedures used to record the postauricular reflex, then highlight the important aspects to consider to adequately measure it, and finally describe and illustrate the signal preprocessing steps and the quantification strategy that can be implemented to analyze the postauricular reflex.

2. Materials

2.1. Electromyography apparatus

1. Amplifier system: A BioSemi ActiveTwo amplifier system (BioSemi Biomedical Instrumentation, Amsterdam, The Netherlands) with a sampling rate of 2048 Hz at direct current (see **Note 2**) is used to collect, amplify, digitize, and store the postauricular EMG signal (bandwidth 0.1 to 417 Hz).
2. Electrodes: Four 4-mm contact diameter Ag-AgCl active electrodes are used, two to measure the postauricular reflex, and the two others serving as a recording reference and ground electrode (see http://www.biosemi.com/faq/cms&drl.htm for further information).

3. Alcohol-imbibed cotton pads: An alcohol-imbibed cotton pad is used to clean the site of the electrode placement and reduce skin impedance before positioning electrodes, while a second alcohol-imbibed cotton pad is used to remove the gel from the electrodes after their use.
4. Electrolyte gel: A commercially available conductive electrolyte gel (SignaGel Electrode Gel®, Parker Laboratories Inc., Fairfield, NJ) is applied on the electrodes to provide a conductive medium between the participant's skin and the electrodes, facilitating the passing of the current from the skin to the electrodes.
5. Wet cotton swabs and distilled water: Wet cotton swabs and distilled water are used to remove the gel from the participant and the electrodes after their use.

2.2. Acoustic probe and audio apparatus

1. Acoustic startle probe: An acoustic startle probe consisting of a 50-ms white-noise burst (105 dB; see **Note 3**) with a nearly instantaneous rise time (< 1 ms) is used to elicit the postauricular reflex.
2. Loudspeakers: Loudspeakers are used to present the acoustic startle probe binaurally (see **Note 4**).
3. Presentation software: MATLAB (version 7.8; The Mathworks Inc., Natick, MA) with the Psychophysics Toolbox extensions ***(23,24)*** is used to control the delivery of the acoustic startle probe (see **Note 2**).

2.3. Data preprocessing and quantification

1. Analysis software: BrainVision Analyzer software (version 2.1; Brain Products GmbH, Gliching, Germany) is used to preprocess and quantify offline the postauricular reflex (see **Note 2**).

3. Methods

3.1. Participant and skin preparation

1. Invite the participant to seat in a comfortable chair (see **Note 5**).

2. Ask the participant to turn off their phone to avoid any possible magnetic interferences with the EMG apparatus and to remove any piece of jewelry (e.g., earrings) that might interfere with the electrode placement.
3. Give the participant an alcohol-imbibed cotton pad and ask them to gently rub it behind their left ear (see **Note 6**) and at the top of their forehead to clean their skin. The goal of this procedure is to reduce the impedance between the skin surface and the electrode gel by removing the oil on the skin surface as well as the dead skin cells ***(25,26)***.

3.2. Electrode preparation and placement

1. Fill the electrodes with the electrolyte gel (see **Note 7**).
2. Ask the participant to pull their left pinna forward and place the electrodes on each side of the tendon of insertion for the postauricular reflex (see **Note 6**). This tendon is easily identifiable in most cases as a fibrous strip that connects the pinna and the scalp ***(10)***. Place one electrode directly posterior to the tendon on the pinna surface and place the other electrode on the scalp over the postauricular muscle ***(3,9,10)*** (see Fig. 3; see **Note 8**). Crucially, the position of the electrodes on the postauricular muscle can significantly impact the recording of the postauricular reflex ***(3)***, which stresses the importance of a consistent and optimal electrode placement across participants.
3. Position the reference and ground electrodes next to each other at the top of the forehead near to the hairline, a site that is relatively inactive electrically ***(25)***.
4. Once the electrodes are placed, check their offset potentials. If the offset is outside the [-40, +40] mV interval (for the BioSemi active electrodes system; see **Note 9**), remove the electrodes, and repeat the skin preparation and electrode placement procedure.
5. Finally, check the EMG signal for noise artifacts (e.g., 50 or 60 Hz interference from power lines; see ref. ***25***) and to ensure its proper recording.

3.3. Experiment design and procedure

1. Once the electrode placement procedure is over, explain the experimental tasks to the participant and give them the instructions.

Fig. 3 Placement of surface electromyography electrodes to measure the postauricular reflex in humans. One electrode is typically placed directly posterior to the tendon of insertion on the pinna surface (red electrode) and the other electrode is placed on the scalp over the postauricular muscle (black electrode).

2. Remind the participant to refrain from moving during the experiment and to look toward the computer screen.
3. In order to habituate the participant to the acoustic probe, start the experiment with the presentation of 10 acoustic startle probes with an interstimulus interval randomly varying between 10 and 20 s (see **Note 10**).
4. After the startle habituation phase is completed, start the experimental task. Here, we describe the specific task used in our previous study (***11***) as an example (see **Note 11**). It consists of an appetitive olfactory conditioning procedure, during which a pleasant odor and odorless air are associated with two different geometric cues (see Fig. 4a), respectively, across three

conditioning phases (habituation, acquisition, extinction; see Fig. 4b). The habituation phase comprises four unreinforced presentations of each geometric cues. During the acquisition phase, each cue is presented nine times, and one cue is systematically paired with the delivery of a pleasant odor (the CS+), whereas the other cue is always associated with odorless air (the CS-). The associations between the cues and the stimulus types are counterbalanced across participants and their order of presentation is pseudorandomized into two different orders. Extinction includes nine presentations of each cue, and the pleasant odor is no longer delivered during this phase. During all the conditioning phases, a trial (see Fig. 4c) starts with the presentation of the cue for a duration of 8 s. The acoustic startle probes are delivered between 5 and 6 s after the cue onset in an equal number of trials for each cue and in approximately 2/3 of the trials (see **Note 12**). The pleasant odor is delivered 6.5 after the CS+ onset during acquisition and released via a custom-made, computer-controlled olfactometer with an airflow fixed at 1 L/min delivering the olfactory stimulation rapidly, without thermal and tactile confounds through a nasal cannula (*27*), for a duration of 1.5 s. An inspiration cue indicating the participant to breathe in evenly is next shown 7 s after the cue onset for 1 s. Each trial is followed by an intertrial interval ranging from 12 to 15 s, during which a fixation cross is presented onscreen (see **Note 13**). Additional acoustic startle probes are delivered between 6 and 7.5 s after the cue offset during 1/3 of the intertrial intervals to decrease their predictability. In total, the task includes 44 trials, ranging from 2 (for the conditions of non-interest during habituation) to 6 (for the main conditions of interest during acquisition and extinction) trials per condition during which an acoustic startle probe is delivered (see **Note 14, Note 15**). The appetitive olfactory conditioning task has a total duration of approximately 16 min. Of relevance for the measurement of the postauricular reflex, there are three important factors to consider in particular: (1) the stimulus duration, (2) the time interval between the stimulus onset and the acoustic probe onset, and (3) the number of trials per condition. It is important to take into consideration sensory effects such as prepulse inhibition and facilitation when determining stimulus duration. The interval between the stimulus onset and the acoustic probe onset should be specifically adapted to whether these phenomena want to be prevented (i.e., by implementing a stimulus duration sufficiently important so that the interval can be long enough to prevent the stimulus from acting as a prepulse) or elicited (i.e., by using an interval enabling the stimulus to act as a prepulse). In addition, enough trials should be included to obtain a reliable estimate of the postauricular reflex magnitude within each experimental condition (see **Note 14**).

5. At the end of the experiment, remove the electrodes from the participant and give them a cotton pad to clean the gel off of their skin.
6. To remove the remaining gel on the electrodes, clean them using a cotton swab and gently pass them under distilled and then carefully dry them (to avoid water electrolysis). Lastly, clean the electrodes with an alcohol-soaked cotton pad.

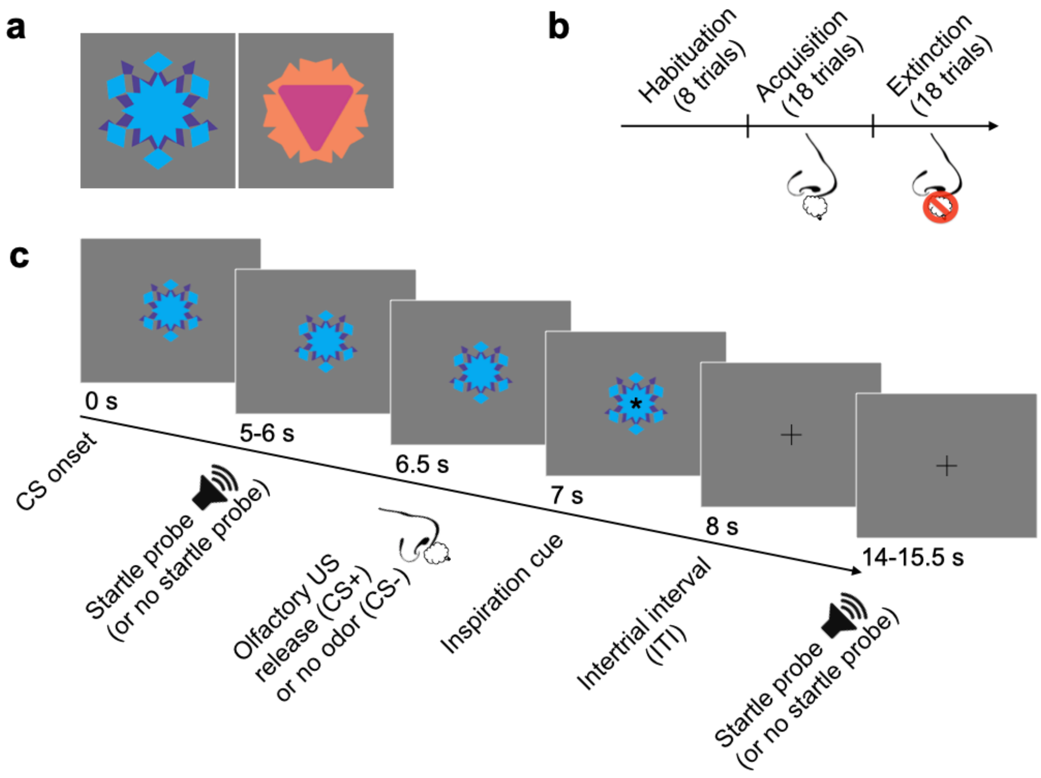

Fig. 4 Illustration of the appetitive olfactory conditioning task. (a) Geometric cues used as conditioned stimuli. (b) Conditioning phases. (c) Trial structure during the acquisition phase. Reproduced from Stussi et al. 2018 ***(11)*** with permission from John Wiley and Sons.

3.4. Acoustic probe delivery

1. In order to synchronize the delivery of the acoustic startle probes with the EMG recording, use the presentation software (e.g., MATLAB) to send triggers (i.e., bytes) via the parallel port to the EMG apparatus each time an acoustic probe is delivered.
2. Be careful to time the triggers to be sent right before the command to deliver the acoustic probe is executed (see **Note 16**). This procedure allows for registering the timestamps of the acoustic probe onsets, which is a critical step given that the postauricular reflex is an extremely rapid reflex ***(4)*** and its analysis is time-locked to the acoustic probe delivery (i.e., event-related analysis).

3.5. Postauricular electromyography signal preprocessing

1. The postauricular reflex signal preprocessing procedure we describe here follows general EMG preprocessing guidelines ***(25,26)***. First, calculate a conventional bipolar montage by subtracting the recorded activity of the electrode placed on the pinna surface from the activity of the neighboring electrode placed over the postauricular muscle (see Fig. 5a,b).
2. Then, filter the raw postauricular EMG signal to minimize noise that is not caused by muscular activity (i.e., low- and high-frequency artifacts that are not comprised within the EMG signal frequency band) and hence increase the signal-to-noise ratio. Specifically, use a digital infinite impulse response band-pass filter (e.g., Butterworth zero-phase filter; 10-400 Hz) (see Fig. 5c; see **Note 17**). If necessary, apply a notch filter (e.g., Butterworth zero-phase filter; 50 Hz) (see Fig. 5d) to reduce noise from alternating current power lines (see **Note 18**).
3. After filtering, rectify the postauricular EMG signal (i.e., the EMG data points are converted to absolute values; see Fig. 5e). The rectification is performed to avoid the negative and positive components of the postauricular EMG signal waveform cancelling each other out during signal averaging ***(25)*** (see **Note 19**).

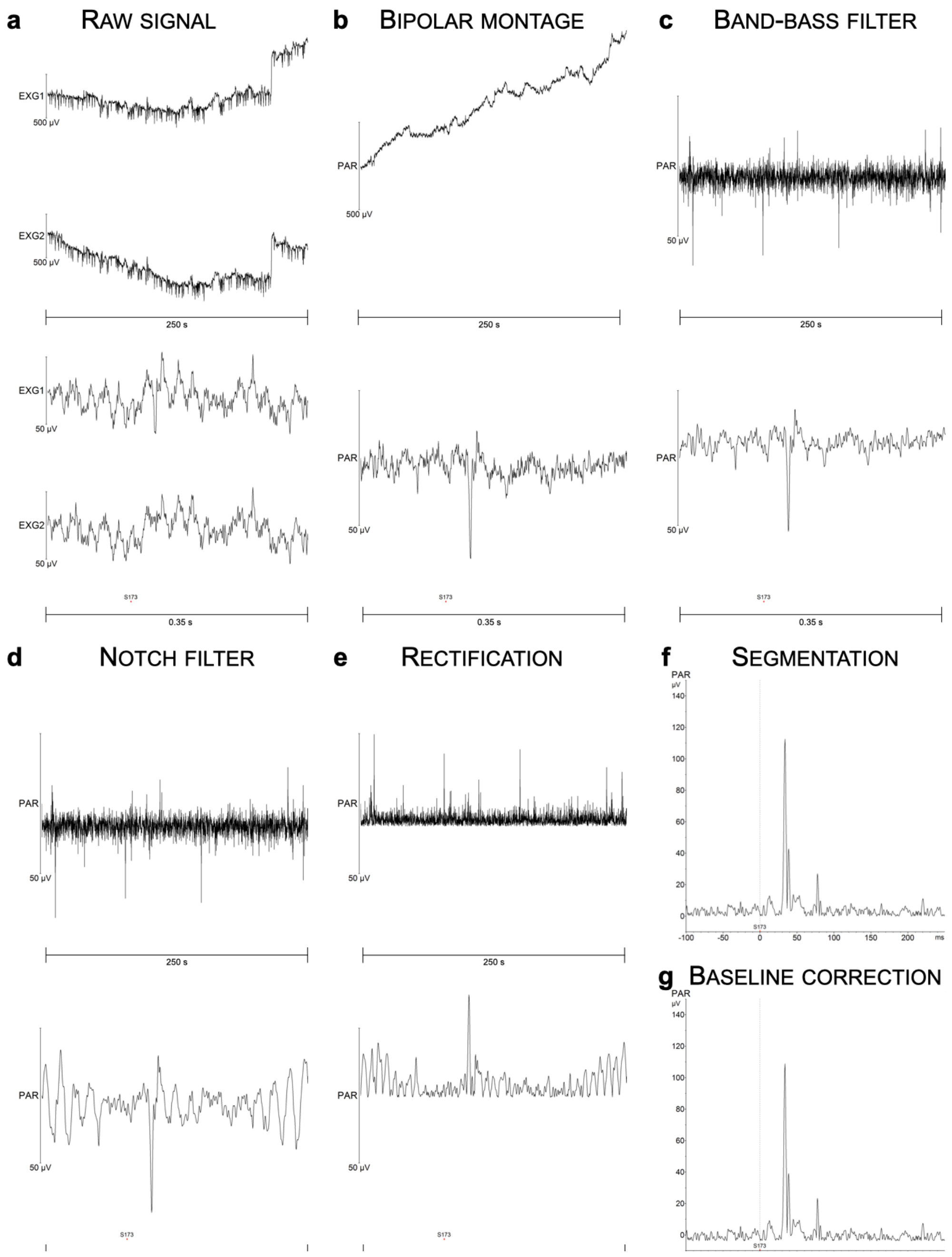

Fig. 5 Postauricular reflex (PAR) signal preprocessing steps. The electromyography (EMG) signal (a) is recorded by two electrodes placed over the postauricular muscle (EXG1) and on the pinna surface (EXG2), respectively. A conventional bipolar montage (b) is calculated by subtracting the recorded activity of the second electrode (EXG2) from the activity of the first electrode (EXG1). The raw postauricular EMG signal is then band-pass (10-400 Hz) filtered (c) and notch (50 Hz) filtered (d). The filtered signal is then rectified (e). Finally, the signal is segmented (f) into epochs starting from 100 ms before to 250 ms after the acoustic probe onset (dotted line), and baseline-corrected (g) with the baseline calculated as the average signal during the 50 ms prior to the acoustic probe onset. For panels a-e, the upper plot depicts the postauricular EMG signal over a period of 250 s, and the lower plot illustrates the signal in response to an acoustic probe within a 350-ms time window.

4. The next preprocessing step consists in segmenting the postauricular EMG signal into epochs starting from 100 ms prior to the acoustic startle probe onset to 250 ms after the probe onset (see Fig. 5f). We suggest that each segment be then baseline-corrected (see Fig. 5g). The baseline can be calculated as the average postauricular EMG activity in the 50 ms preceding the acoustic startle probe onset. Visually inspect all segments and remove by hand segments that are identified as containing excessive baseline shifts (in our study, 96 out of 2310 trials were discarded on this basis, which corresponds to 4.16% of the trials) from the analysis (see **Note 20**).

3.6. Response quantification

1. Given its low signal-to-noise ratio as a microreflex, we suggest quantifying the postauricular reflex by averaging the signal of the rectified waveforms ***(4,8-10,12,14-17)***. For each individual participant (**Note 21**), we recommend averaging the preprocessed postauricular EMG segments across trials within each separate experimental condition (see **Note 22**).
2. Score the postauricular reflex magnitude on the resulting aggregate waveform as the baseline-to-peak amplitude for each condition. The peak amplitude is computed as the maximum postauricular EMG activity that occurred within a 5-35 ms time window following the acoustic startle probe onset (see **Note 23**).
3. Peaks can be detected automatically using BrainVision Analyzer, but it is important to carefully check them manually afterward. The raw unstandardized extracted peaks (in µV) can for instance be used in the data analysis (see **Note 24**).

To illustrate the modulation effects of the postauricular reflex that are typically found, Fig. 6 depicts the resulting grand-averaged (i.e., averaged across participants) rectified postauricular reflex waveforms for attentional and affective modulation. Specifically, Fig. 6a illustrates the inhibition of the postauricular reflex during a visual foreground stimulus compared to during intertrial intervals where only a fixation cross was presented, thereby reflecting attentional modulation of this reflex by continued attention during image processing. Fig. 6b shows the effects of affective modulation of the postauricular reflex, as indicated by a higher postauricular reflex amplitude during the presentation of the geometric cue associated with a pleasant odor than during the presentation of the geometric cue associated with odorless air during appetitive olfactory conditioning.

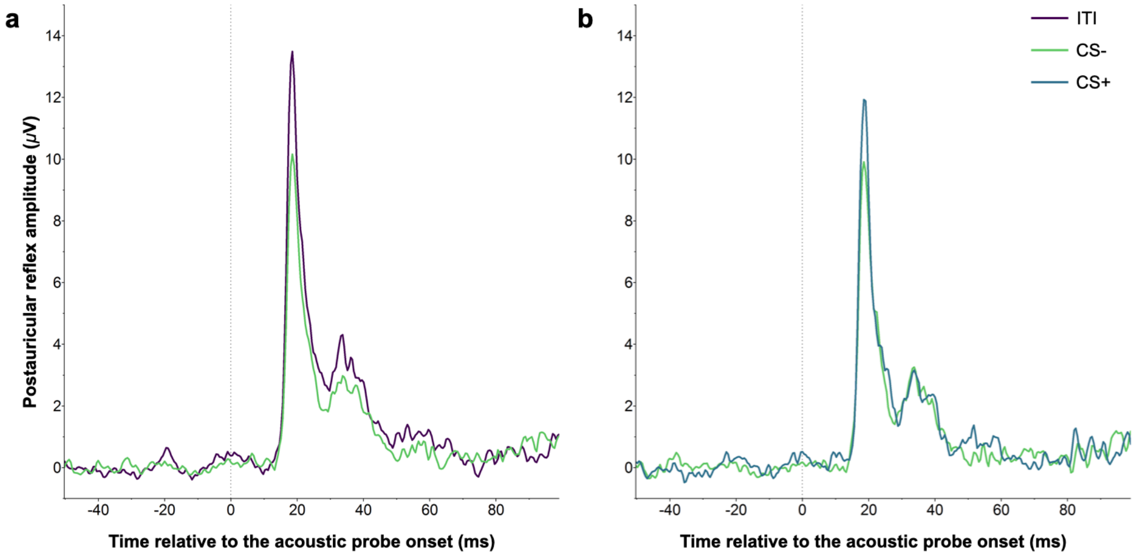

Fig. 6 Modulation effects of the postauricular reflex. (a) Grand-averaged postauricular reflex waveforms (14 trials by 55 participants) in response to a 50-ms white-noise acoustic probe at 105 dB during intertrial intervals (ITI) versus the presentation of a geometric cue associated with odorless air (CS-). These results illustrate the inhibition of the postauricular reflex during image processing. (b) Grand-averaged postauricular waveforms (6 trials by 55 participants) in response to a 50-ms white-noise acoustic probe at 105 dB during the presentation of a geometric cue predicting the delivery of a pleasant odor (CS+) versus a geometric cue associated with odorless air (CS-). These results show the affective modulation of the postauricular reflex, as reflected by a higher amplitude when viewing a stimulus associated with an appetitive outcome compared to a neutral stimulus. Adapted from ref. ***(11)***.

Finally, Fig. 7 further illustrates the large variability in the postauricular reflex that can be observed across individuals by showing the average postauricular reflex amplitude during the intertrial intervals separately for each participant.

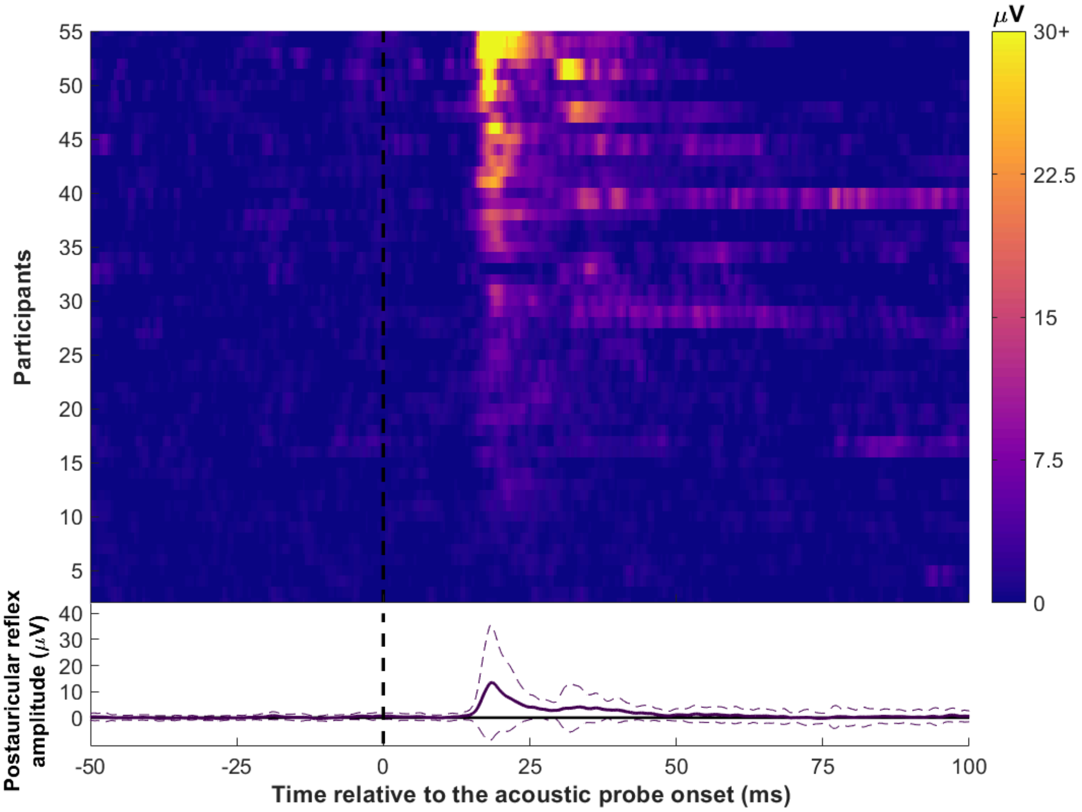

Fig. 7 Interindividual variability in the postauricular reflex. Upper panel: Ranked individual averaged postauricular reflex amplitude in response to a 50-ms white-noise acoustic probe at 105 dB during intertrial intervals across 55 participants (14 trials). The amplitude was calculated as the maximum voltage within the 5-35 ms time window after the acoustic probe onset (vertical dashed line). Lower panel: Grand-averaged postauricular reflex waveform during intertrial intervals. The solid lone represents the average postauricular reflex amplitude across participants and the dashed lines represent ± 1 standard deviation.

4. Notes

1. The postauricular reflex is often measured at the same time as the startle eyeblink reflex because these two reflexes can be elicited in response to the same acoustic startle probes and require the same materials to be recorded. Some authors describe the postauricular reflex as a component of startle--as is the eyeblink reflex--and more specifically as a vestigial startle response ***(5)***. Of particular interest, the postauricular reflex furthermore presents an opposite pattern of modulation to the startle eyeblink reflex, the latter being potentiated in response to aversive stimuli compared to neutral stimuli and, in some cases, inhibited in response to

appetitive stimuli ***(28)***. For this reason, the postauricular and the startle eyeblink reflexes are often considered as being complementary in that they can be used to index appetitive and aversive processing, respectively.

2. The specific equipment described here corresponds to the one we used in our study ***(11)***. However, other EMG recording systems, sampling rates (though a sampling rate of a least 1000 Hz is generally recommended; see ref. ***25,26***), stimulus presentation software, and analysis software can be used depending on their availability and the researcher's preference.
3. The postauricular reflex can be evoked by both soft clicks and loud noises ***(8)***; however, the postauricular reflex amplitude increases with the acoustic probe's intensity or volume ***(6,29)***. Accordingly, this aspect should be considered when selecting the intensity of the acoustic probe and weighed against a heightened aversiveness to louder noises, while making sure the participant's hearing safety is not compromised. Another element to consider is the level of background noise in the testing environment, which may act as a prepulse (or alternatively mask a prepulse) ***(25)***. Therefore, it is important that the participant be isolated from environmental noises to control for unwanted influences thereof.
4. Note that most of the studies investigating the postauricular reflex typically used headphones to deliver the acoustic probes ***(4,7,8,10,12-14,17-19)***. The calibration of the sound intensity is usually easier to accomplish with high precision using headphones rather than loudspeakers ***(25)***. Headphones might also be preferred in laboratory environments where the delivery of loud noises can be problematic. By contrast, loudspeakers are preferable when headphones might interfere with the electrodes or other sensors ***(25)***.
5. Although it has been reported that an upright posture increases background activity in the postauricular muscles ***(4,14)***, participants should ideally feel comfortable enough to be able to perform the entire study with minimal neck and eye movements. In fact, flexing the neck forward ***(4)*** or tucking the chin toward the neck ***(30)*** have been reported to increase the postauricular reflex amplitude. Similarly, rotation of the eyes toward the sound-eliciting source has been shown to amplify the postauricular reflex and has been suggested to represent a critical factor that can account for a large part of the inter- and intraindividual variability

found in the measurement of this reflex ***(30)***. If visual stimuli are used during the experiment, adjusting the chair height is recommended so that the participant's gaze coincides with the center of the computer screen when possible. To control for eye movement in studies that do not use visual stimuli, it may be worth considering using a blank screen with a fixation cross that participants can fixate on when stimuli are presented. A supplementary option to mitigate the possible impact of eye rotation is to record eye movements via (vertical and horizontal) electro-oculography and exclude from the analysis trials that are contaminated by excessive eye movements.

6. In most cases, psychophysiological studies measuring the postauricular reflex recorded the postauricular muscle EMG activity from both ears ***(3,4,7-10,12,14,15,17)***, and typically, the signal is averaged across them. Interestingly, no systematic difference has generally been observed when comparing the postauricular reflex of the left versus the right ear ***(12)***.
7. It is particularly important to pay attention that no air bubbles are introduced when filling the electrodes with the electrolyte gel. Such air bubbles can impede the conductive power of the gel, which may eventually interfere with the EMG measurement. To that end, the use of a plastic syringe is suggested. First, fill the syringe with the gel and then slowly push the plunger until the air is out of the syringe, while using a tissue to wipe off the excess gel coming out of it. This should help remove the air bubbles inside the gel before it is eventually injected into the electrodes. It is also important to avoid overfilling the electrodes with gel and to wipe off the excess gel remaining on the skin surface after the electrode placement to prevent the residual gel from creating a conductive bridge between the two electrodes, which may weaken or eliminate the recorded EMG signal ***(25)***.
8. Because the postauricular reflex is measured as a difference in electric potential, it is recommended to consistently use the same site placement for each respective electrode (i.e., the first electrode is always placed on the scalp over the postauricular muscle, and the second electrode is always placed posterior to the tendon on the pinna surface, or vice versa).
9. For passive electrode systems, the electrode impedance should be checked and should preferably be below 5 kΩ ***(25)*** or below 10 kΩ as the maximum upper limit.

10. Because the postauricular reflex is resistant to habituation (i.e., its amplitude does not decrease across time ***(29)*** or the number of acoustic probes presentations ***(7)***), the inclusion of a startle habituation phase is not mandatory but may nonetheless help participants get used to the probe. The inclusion of such a habituation phase is highly recommended when the postauricular reflex is measured at the same time as the startle eyeblink reflex to reduce initial reactivity of the latter.
11. The information provided here is to be merely taken as an illustration of an experimental task where the postauricular reflex is measured as an indicator of appetitive processing. For the readers interested in measuring the postauricular reflex in their own study, the task design and specifics will of course depend on the nature of the experiment and the research question.
12. In case the trial-by-trial trajectory of the postauricular reflex is central to the research question, it is suggested that each trial be probed. However, such a type of analysis might not be generally recommended because of the low signal-to-noise ratio of the postauricular reflex ***(4,9)***.
13. The relatively long stimulus presentation duration in our procedure was implemented because of the concurrent recording of electrodermal activity ***(11)***, which can be affected by the acoustic startle probe when the time interval between the stimulus onset and the probe is too short. Moreover, a long intertrial interval was used to allow the electrodermal activity--which is relatively slow--to return to a baseline level before the start of the next trial. Shorter stimulus presentation (e.g., 6 s), stimulus onset-acoustic probe onset interval (e.g., 3-5 s), and intertrial interval (e.g., 3 s) durations ***(8,10,12,17)*** can be implemented when only the postauricular reflex (or a combination of the postauricular and startle eyeblink reflexes) is measured.
14. Although the minimal number of trials required to obtain a stable and reliable measure of the postauricular reflex remains to be established, it has been suggested that including at least 12 trials per condition appears to produce a robust estimate of the postauricular reflex ***(31)***.
15. The decision to include 6 trials per experimental condition of interest was based on typical Pavlovian conditioning procedures used in the field ***(32)*** and constraints related to the overall

duration of the experiment. But it is recommended to include as many trials per condition of interest as possible whenever it is warranted and feasible.

16. It is crucial to assess the potential occurrence of any time delays between the moment the trigger is sent, the moment the command to deliver the acoustic probe is executed, and the actual acoustic probe onset. It is strongly recommended to check this aspect before starting data collection. If a fixed time delay is detected, this delay should be considered and corrected (e.g., by systematically recoding the triggers) after data collection to appropriately score the postauricular reflex. In the event the delay between the command and the onset is variable, an option is to record the sound delivered through the loudspeakers (or headphones) using a microphone and to subsequently use this recording to detect and appropriately code the acoustic probe onsets for analysis, which is especially important when using signal averaging.
17. Across the literature, different band-pass filter ranges have been used to filter the postauricular EMG signal, typically varying from 3-300 Hz ***(14,15)*** to 0.05-1000 Hz ***(13)***. Importantly, the postauricular muscle has been shown to produce electrical activity that has frequency components primarily in the 25-300 Hz range ***(3)***.
18. Note that the power line frequency is typically 50 Hz in large parts of the world (Europe, most of Africa, most of Asia, and Oceania); by contrast, it is typically 60 Hz in North and Central America, parts of South America, and parts of East Asia. The use of a notch filter is generally not recommended when the EMG signal can be recorded under conditions that minimize 50/60 Hz power line interference ***(25)***. This is because notch filters have the disadvantage of removing noise in the 50 or 60 Hz range and also true EMG signal ***(25)***.
19. In contrast with the startle eyeblink reflex ***(25)***, the postauricular reflex signal is generally not smoothed (e.g., by using a low-pass filter) after rectification.
20. While trial exclusion based on visual inspection involves an inherent subjective component, an inter-judge reliability approach can be implemented to mitigate this aspect. This approach consists in having multiple researchers perform the visual inspection to determine which trial exclusions are agreed upon. Additionally, it is strongly recommended that the criteria adopted for rejecting a trial always be reported, along with the percentage of excluded trials using

these criteria ***(25)***. A rejected trial should furthermore not be considered equivalent to a trial in which there is no response or wherein no distinguishable response can be detected ***(25)***.

21. It should be noted that there exists a substantial variability in the postauricular reflex both across (see Fig. 7) and within individuals ***(33)***. Moreover, a study reported an absence of a measurable postauricular reflex in at least one ear in 32% of their sample and bilaterally in 7% of their sample ***(34)***. On an anatomical level, the postauricular muscle has been reported to be present in 95% of the studied population ***(35)***. As previously mentioned (see **Note 5**), it has, however, been argued that a large portion of this individual variability may stem from the influence of eye rotation on the measurement of the postauricular reflex ***(30)***.
22. Although signal averaging is generally needed and recommended to reveal the postauricular reflex ***(6)***, some studies have scored the postauricular reflex using a trial-by-trial approach ***(18,31)***.
23. Various time windows have been used to score the postauricular reflex magnitude. In general, these time windows are situated within an interval ranging from 5 to 50 ms following the acoustic probe onset ***(8,10-13,16-19)***.
24. Due to the large interindividual variability in the postauricular reflex, the postauricular reflex magnitudes are sometimes standardized within participants across conditions or trials (e.g., using Z-scores or T-scores) before data analysis ***(8,12,13,31)***.

5. Conclusions

In this protocol, we reviewed and described how the postauricular reflex can be measured and quantified in humans. Our goal was to provide information on the materials and methods that can be used to record the postauricular reflex and illustrate the preprocessing and scoring of the recorded signal. We also aimed to demonstrate the value of the postauricular reflex as a psychophysiological tool for the study of appetitive motivation processes, thereby highlighting its relevance for food science.

Nonetheless, it is important to note that the affective modulation of the postauricular reflex by appetitive stimuli has mainly been investigated during the presentation of visual stimuli usually considered as pleasant that either consist in representations of a food reward (e.g., food images) or are associated with a food reward. Accordingly, it remains to be established whether such effects likewise occur during the consumption of the food reward per se. An interesting avenue for future research would therefore be to examine whether the postauricular reflex in response to an acoustic probe is potentiated during the presentation of pleasant olfactory or gustatory (e.g., tastes) stimuli, and whether these affective modulation effects are similar or even larger than those observed during the presentation of appetitive visual stimuli. In that respect, we hope that the present protocol will contribute to sparking further interest in the use of the postauricular reflex as a psychophysiological index of appetitive motivation, which could ultimately foster insights into the psychological and physiological mechanisms underlying food reward processing in humans.

6. References

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