Reciprocal modulation of eye-blink and pinna-flexion components of startle during reward anticipation

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Abstract

Because expectancies play a central role in current theories of dopaminergic neuron function, it is important to develop measures of reward anticipation processes. In the present study, reflexogenic bursts of white noise were presented to 39 healthy young adults as they awaited rewards and punishments in a gambling-like task. The rewards were small pieces of chocolate; the punishments, segments of bitter-tasting banana peel. Consistent with prior research on affective valence, postauricular reflexes were larger prior to rewards than punishments, whereas the reverse was true for acoustic blink reflexes. We theorized that potentiation of the postauricular reflex prior to consuming appetizing food is related to the priming of ear-retraction musculature during nursing in our remote ancestors.

Descriptors: Startle, Post-auricular reflex, Reward, Attention

Understanding the reward system has become a major goal of neuroscience research due to the involvement of this system in addiction, obesity, pathological gambling, depression, and Parkinson’s disease. The present study is one of a series intended to advance that understanding by developing measures of anticipatory attention toward rewards (e.g., Hebert, Valle-Inclán, Oh, Rolan, & Hackley, 2006; Mattox, Valle-Inclán, & Hackley, 2006; Munoz, 2007).

Developing an electrophysiological index of reward anticipation is critical because some types of implicit learning appear to be based on the difference between anticipated and received reinforcement (Rescorla & Wagner, 1972). According to the most widely accepted theory of the reward system (Schultz, 1998; Schultz, Dayan, & Montague, 1997) mesencephalic dopamine cells broadcast a diffuse teaching signal that encodes the onset of reward-predicting events, as well as the amount of reward that is received relative to the amount that was expected. For example, if a food reward is received when none was expected, the cells’ discharge rates increase. This briefly enhances plasticity at target synapses (e.g., at conjunctival D1-NMDA synapses; Baldwin, Sadeghian, & Kelly, 2002).

Reinforcement-Based Learning in Gambling Tasks

An earlier experiment at this laboratory illustrates the task to be employed as well as its sensitivity to dopamine and reward incentive level (Mattox et al., 2006). The study involved recording a motivationally sensitive event-related potential, the Stimulus Preceding Negativity (SPN), in Parkinson’s patients who had been withdrawn overnight from L-dopa. Patients and control participants performed a probabilistic classification task in which 1–3 cards with geometric designs were displayed on each trial (Knowlton, Mangels, & Squire, 1996). After studying the cards, they pressed one of two computer keys to indicate their prediction regarding the outcome for that trial, “sunny” or “cloudy.”

In this trial-and-error learning task, the various cards are related to trial outcome in a probabilistic rather than deterministic manner, as in games of chance. For example, a card with purple circles might predict sunny weather with a probability of .57 and cloudy weather with the complementary value of p = .43. The optimum response would be “sunny,” but this response will sometimes be followed by punishment rather than reward. When multiple cards are shown, the implicit probabilities are to be averaged for the optimal response. A feedback display indicating whether the subject’s guess is right or wrong and the money won or lost is presented after a delay of a few seconds so as to allow anticipatory processes to be fully manifest.

In neurologically normal subjects, a pronounced SPN developed during this interval between key press and feedback. Consistent with the hypothesis that parkinsonian individuals are impaired at reward anticipation due to loss of dopaminergic neurons, the SPN was greatly attenuated in patients with an early...
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compared to 5 a cigarette break as rewards in the weather prediction task. Following receipt of points, acoustic probes evoked larger postauricular reflexes and smaller eye-blink reflexes as compared to punishment trials, which simply delayed the smoking break. In the present study, we used bits of chocolate as rewards for self-described “chocoholics” who had gone without sweets for at least 12 h. As punishment for incorrect responses, participants were required to chew and then expiate small segments of banana peel.

Because these positive and negative stimuli were in the same modality—gustatory/tactile—the attentional confound characterizing the Skolnick and Davidson study was avoided. In their experiment, rewards were visual (display of monetary winnings) whereas both punishments and reflexogenic probes were auditory (white noise bursts). Some previous studies have shown that acoustic and cutaneous blink reflexes are suppressed when attention is directed toward a competing modality (e.g., Hackley & Graham, 1984). Although the generality of this phenomenon has been convincingly challenged (Lipp, Siddle, & Dall, 1998), the possibility remains that acoustic reflexes were suppressed by attention during receipt of rewards in the Skolnick and Davidson study as participants contemplated the visual display.

The second goal of our study was to extend recent findings that activation of the postauricular muscle during acoustic startle is enhanced while viewing pleasant rather than unpleasant slides (Benning, Patrick, & Lang, 2004; Hess, Sabourin, & Kleck, 2007). In most mammals, this rapid (onset 9–15 ms) component of startle pulls the pinnae back as a protective measure, similar to closing the eyes, as the organism braces for attack. In humans and most apes, however, the ears are permanently pulled back against the head, so the response has become entirely vestigial. No physical movement occurs, even in people who have the ability to voluntarily wiggle their ears (Hackley, Woldorff, & Hillyard, 1987). Confirmation of an inverse pattern of modulation for two components of the same reflex, eye closure and pinna retraction, would call for a revision of accepted theory.

Methods

Participants

The final sample comprised 39 young adults (mean age = 18.8 years, standard deviation = 1.0) who were recruited from an introductory psychology class. Twenty-five were women, 14 were men; all signed a consent document that had been approved by the campus ethics committee. The data from 4 additional subjects were rejected, 3 because of technical problems, 1 because of illness.

The chocolate lovers were recruited via targeted announcements on the department’s Web-based system. In a postexperimental questionnaire, 74% of participants said that they eat chocolate at least two times a week. On a scale ranging from 1 (“I don’t like chocolate”) to 9 (“I love it”), they gave a mean rating of 7.7 (SD = 1.1). Participants were asked to refrain from eating chocolate or other sweets for 12 h and food of any kind for 2 h prior to the start of the experiment. Responses on the postexperimental questionnaire indicated good compliance.

Apparatus and Stimuli

Because an upright posture enhances background activity in the postauricular muscles, participants were seated in an office chair with minimal back support and no arm rests. They faced a com-
puter monitor (37-cm diagonal) positioned on top of a pair of adjacent wooden boxes, 39 cm deep, with 17-cm square openings under the face of the monitor. In one of these dark recesses lay a dish with pieces of various types of chocolate. The dish within the other recess held \( \sim 1.5 \text{-cm squares of banana peelings that had been lightly marinated in lemon juice to prevent discoloration. The top of each box supported a small incandescent bulb, which illuminated the selected food for that trial. A small plastic bag hung on the right side of the table. On error trials, participants spat the bad-tasting but nontoxic segments into the sack after chewing them for about the same amount of time as they did the chocolate, on correct-response trials.

The reflexogenetic stimulus was an unrampered white-noise burst with a duration of 50 ms and an intensity of approximately 95 dB (SPL-A) presented over light-weight stereo headphones (Sony, model MDR-25). Artificial ear couplers for ordinary headphones are not available; hence, calibration was only approximate. Voluntary responses in the gambling-like task were entered on a computer keyboard.

Procedure

We used Knowlton’s weather prediction task (Knowlton et al., 1996) because it requires reinforcement-based implicit learning, yields a predictable ratio of correct to incorrect responses, and permits comparison with our previous reward-anticipation studies (Mattox et al., 2006; Hebert et al., 2006). The trial structure was as follows: Subjects viewed a display of 1–3 cards for as long as they liked and then pressed the F1 or F12, top-row key to indicate their guess for that trial, sunny or cloudy. The card display disappeared immediately. Four seconds later, a feedback display showing a sun or rain cloud was presented for 2 s, accompanied by the word “Correct” or “Incorrect.” The appropriate food bin was illuminated and subjects directed their gaze at it. Between 3 and 5 s after offset of the feedback display, the white noise burst was presented. This startle probe was delivered on a random 80% of the trials. Six seconds after the feedback display had been presented (1–2 s after the probe), the monitor displayed the command “OK, eat now.” This display had large letters and a bright background so that it could be noted with peripheral vision. Subjects were given 12 s to consume the food before the next trial began. Probes were not presented during the intertrial interval because this period does not provide a stable, meaningful control condition with which to isolate affective or cognitive processes (Gusnard & Raichle, 2001).

The general procedure was similar to that of the typical psychophysiology experiment: Participants were oriented to the task and recording procedures; they signed an IRB-approved consent form; electrodes were attached to their face and scalp; after 16 practice trials they performed the experimental task (~65 min); the electrodes were removed; subjects then filled out a brief questionnaire and were dismissed with thanks. The experiment consisted of 147 trials arranged in 3 blocks of 49 trials between which were brief rest periods. There were no across-block or group manipulations. The only comparison was between trials with correct and incorrect responses, which were followed by reward or punishment, respectively.

Data Acquisition and Analysis

Electromyographic (EMG) activity was recorded from the right orbicularis oculi and both the left and right retrahens auriculam muscles. Following the method described by Sollers and Hackley (1997), retrahens auriculam was identified by pulling forward on the pinna until its tendon was visible as a wedge-shaped protrusion overlying the mastoid process. One electrode was taped to the skin on or immediately posterior to the tendon. The other bipo-
larly configured electrode was attached just behind the first, presumably over the belly of the muscle. Lower eyelid EMG recordings were congruent with published guidelines (Blumenthal et al., 2005). The bandpass for the 4 EMG recordings was set between 3 and 300 Hz; analog-to-digital conversion was carried out at a rate of 600 Hz; electrode-skin impedance did not exceed 5 kΩ. There was no supplemental filtration, smoothing, or integration.

The continuous EMG recordings were segmented off-line into epochs extending from 50 ms prior to probe onset until 250 ms later. After cleansing the data of trials with blinks-in-progress at the time of probe onset, motion artifact, and amplifier blocking, a correction was applied to compensate for differential habituation across conditions. The concern was that, because subjects commit more errors as they begin learning the task, trials with punishments should occur on the average earlier in the session than trials with rewards. This could therefore cause reflexes on reward trials to artifactually appear smaller, due to habituation. To compensate for this bias, error trials at the beginning of each block and correct-response trials at the end of each block were rejected until the average ordinal position for the two were equated. The resulting sample comprised 75% of the original pool of trials (4,158 trials, summed across 39 participants). In future studies, we plan to evaluate more efficient approaches (e.g., analysis of covariance).

Only trials in which reflex EMG was at least two standard deviations above background noise levels were retained for signal averaging. The window for these signal/noise tests extended from 50 to 110 ms for orbicularis oculi and 13 to 50 ms for retrahens auriculam. In previous research at this laboratory (e.g., Sollers & Hackley, 1997), all trials that were free from artifact were used for signal averaging. This strategy is standard in EEG research and has been adopted as the recommended method for signal averaging of startle EMG (Blumenthal et al., 2005). However, for the present study we directly compared the “average amplitude” versus “average magnitude” approaches and found that the former (i.e., including only responses that were larger than the background activity) yielded a better signal-to-noise ratio.

For the postauricular reflex (PAR), 34 participants had adequate responses for at least one ear. Responses from the ear with the largest amplitudes were included in the statistical analysis.

1In addition to assessing valence-specific anticipatory states, we had intended to compare reflex modulation and event-related potential (SPN) effects. This goal was not realized because, as subjects looked at and prepared to consume the food, their electroencephalographic (EEG) activity was obscured by large (50–300 μV), slow potentials that were comparable in size and morphology at all scalp and periorbital sites. The pattern was similar to that of the familiar gum-chewing artifact, although less rhythmic and without obvious masseteric EMG. Consequently, we assumed that these voltage fluctuations reflected a conditioned salivatory response in conjunction with small intraoral movements that modulated the standing glossopharyngeal potential. The relevant loadings could not be identified for exclusion using Independent Component Analysis (ICA). Similarly, an analysis of event-related desynchronizations in the alpha and beta bands failed to yield usable data. Given the pressing societal need for research on obesity and eating disorders, it will be im-
portant for future psychophysiological studies to develop methods for isolating and removing this artifact.
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The proportion of accepted trials for PAR was 34% (1,062 trials, summed across 34 subjects). Blink reflexes were only recorded from the right eye, with 36 participants manifesting sufficiently large responses. The proportion of trials surviving for size and absence of artifacts was 47% (1,466, across 36 subjects). Statistical analysis consisted of Student t-tests for peak amplitude and for mean amplitude averaged across the measurement window.

Results

Validation Checks
As noted earlier, people who like chocolate were specifically recruited for this study. They were self-deprived of sweets for at least 12 h and of food in general for at least 2 h before arriving at the lab. Participants consumed an average of 84 pieces of chocolate (about 5 mm diameter), and sometimes spontaneously commented at the end of the experiment that they were sated. Nonetheless, they gave strongly positive evaluations of these sweets. When asked, “How much money would we had to pay to equal the value of one piece of chocolate?” the mean response was $1.15. In contrast, subjects reported that they would have paid us $1.03 on error trials not to have had to chew on the banana peel segments. The corresponding standard deviations were $1.59 and $1.50, respectively.

To our knowledge, no one has ever asked people how much they would be willing to pay to look at or to avoid looking at slides in the International Affective Picture System (IAPS). So, in order to relate our findings to previous studies, we asked participants to give post hoc ratings using the Self-Assessment Mannequin (SAM) scale, which is standard in the field of emotion research (Center for the Study of Emotion and Attention, 1988). The mean scores for chocolate and banana peel on the 9-point SAM scales were, respectively, 7.8 and 2.5 for valence, 5.5 and 4.2 for arousal, and 5.5 and 4.7 for dominance. The corresponding standard deviations were 1.5 and 1.2 for valence, 2.2 and 2.4 for arousal, and 2.0 and 2.4 for dominance.

Task performance was in the expected range for neurologically normal individuals (Knowlton et al., 1996; Mattox et al., 2006). The percentage of trials in which the participant guessed the outcome with the highest likelihood of being rewarded averaged 59.0% (Blocks 1, 2, and 3 = 57.8, 57.8, and 61.5%, respectively). This task can only be learned by trial-and-error, so the participants clearly did make use of the positive and negative feedback.

Reflex EMG
As shown in the grand average waveforms of Figure 1, reflex amplitudes conformed to predictions. Eye-blink EMG was larger when a punishment rather than a reward was about to be received (peak amplitude, 39.8 vs. 30.8 μV (SD = 13.3); t(35) = 4.04, p < .001; mean amplitude, 15.2 vs. 11.7 μV (SD = 5.39); t(35) = 3.80, p < .001; Cohen’s d = 0.25). For the postauricular reflex, the opposite was true; EMG amplitudes were larger prior to rewards than punishments (peak amplitude, 20.0 vs. 15.5 μV, SD = 7.8; t(33) = 3.36, p < .003; mean amplitude, 6.86 vs. 5.65 μV, SD = 1.9; t(33) = 3.53, p < .001, Cohen’s d = 0.19).

The reciprocal relationship between the two reflexes was assessed in two ways. One method comprised a within-subjects ANOVA with a 2 × 2 factorial structure, reward/punishment by orbicularis oculi/retrahens auriculam. The interaction of these factors was highly significant, F(1,30) = 16.03, p < .001. The second approach was correlational. An across-subjects correlation of the valence effect for eye blink and pinna retraction (mean amplitude on reward trials minus punishment trials) did not reach significance (r(31) = −.222, p = .229), nor was the size of these two components of startle correlated (e.g., mean amplitude on reward trials, r(31) = −.006, p = .976). Interestingly, participants who had strong feelings about the food (valence rating for chocolate minus banana peel) had small postauricular reflexes, r(34) = −.523, p < .002 (reward trials; punishment trials were about the same), whereas there was no such relationship for blink, r(36) = −.09, p = .603.

Discussion
The results showed a reliable modulation of startle reflex amplitude during anticipation of rewards and punishments that was in the predicted direction. This conflicts with the prior null results obtained by Skolnick and Davidson (2002) in their three experiments. Similarly, prior research in which subjects anticipate pleasant versus unpleasant slides have failed to document significant valence effects (e.g., Mallan & Lipp, 2007; Sabatinelli, Bradley, & Lang, 2001).

It is not entirely clear that our positive results were due to the use of a stronger manipulation. Subjects in the Skolnick and Davidson study won an average of $1.02 on reward trials, which is similar to the $1.15 our hungry subjects estimated they would have paid for each piece of chocolate. Our punishments may have been a bit more aversive: Participants said they would have paid $1.03 to avoid eating each banana peel segment, whereas the
aversive stimuli used by Skolnick and Davidson—white noise bursts—were not very different than the startle probes that occurred as equally often on reward as on punishment trials. In the Sabatinelli et al. study, young men who were exceptionally afraid of snakes failed to exhibit valence-specific blink modulation as they awaited slides of scary snakes, household objects, and mildly erotic scenes. Neither SAM nor monetary equivalent ratings were reported, but intuition suggests that these values might have been comparable to the ones obtained in our study.

Studies of rats have reported that anticipation of food rewards (e.g., Baschnagel, Hawk, Colder, & Richards, 2007; Schmid, Koch, & Schnitzler, 1995) or electrical stimulation of the reward pathway (Steidl, Li, & Yeomans, 2001) attenuates the size of the startle reflex. The discrepancy between our results and the previous studies noted above might be due to our use of natural rewards and punishments (unlike money or photos) or the fact that these reinforcers were consequences of skilled action (unlike lottery guesses).

Another possibility is that our positive findings reflect the emotional state following receipt of feedback rather than anticipation of the reward or punishment. The follow-up study mentioned earlier (Munoz, 2007) is relevant here. Participants in that study did not always receive a reward—points toward a cigarette break—when they were correct. The display indicating whether points were or were not awarded occurred 7.5 s after feedback. Startle probes were then delivered 2.5 to 3.9 s after onset of this slide. Significant potentiation of the postauricular reflex and suppression of the blink reflex occurred only on reward trials. If the pleasure of receiving positive feedback following a correct response generated the modulatory effect, this blink suppression and PAR potentiation should also have been obtained on correct but unrewarded trials.

It is something of a puzzle that two components of the same reflex should be modulated in opposite directions by emotion. Benning and colleagues (2004) speculated that attentional orienting might be involved. However, an attention effect on the afferent limb of the startle circuit might be expected to affect eye blink and pinna retraction components similarly (Hackley, 1993). Also, the effect should be directionally specific, with retraction of the ears only when orienting to a stimulus behind the head (Stekelenburg & van Boxtel, 2001). Finally, as the authors noted, attentional orienting should be elicited during negatively as well as positively valent stimuli.

Johnson, Geary, and Hackley (in preparation) offer a new account of why the postauricular component of startle exhibits a pattern opposite that of the eye blink component. They propose that the normal suppression of defensive reflexes during appetitive states is eclipsed by a reverse bias that is specific to the pinna musculature. All infant mammals that can move their ears pull them back during nursing. This signals the infant’s intent to nurse and gets the ears out of the way so that the head can be comfortably positioned. Natural selection may, therefore, have created circuits that prime ear-retraction muscles when either breasts or appetizing food are viewed. Consistent with this account, our results showed that postauricular reflex amplitudes are larger prior to consumption of appetizing than distasteful foods. Our theory is also supported by a recent detailed analysis of modulatory effects as a function of slide type (Sandt, Sloan, & Johnson, 2009). Across ten categories of emotionally provocative slides, only two types yielded potentiation of the postauricular reflex relative to neutral control trials—food and erotica.

Because they are reciprocally modulated, the combination of eye-blink and postauricular reflexes offers a special advantage in ruling out confounds. Most factors that might bias the results of an affective reflex modulation study should influence the two responses in the same direction. Visual attention and prepulse inhibition, for example, might be expected on a priori grounds to reduce the magnitude of both components, whereas one might suppose that auditory attention, non-specific arousal, and increases in general muscle tension would facilitate both components (reviewed in Hackley, 1993). Consequently, the $2 \times 2$, cross-over interaction of valence $\times$ muscle could constitute an unambiguous signature for the effect of emotion.

Psychophysicists had at one time hoped that the contrast between spontaneous EMG activity in the zygomaticus (smile) and corrugators (brow-knitting) muscles would offer such a possibility (Cacioppo, Petty, Losch, & Kim, 1986). Unfortunately, it was later noted that zygomaticus can also be engaged during negative emotions, such as those accompanied by grimaces and smirks (e.g., Mattox et al., 2006). If blink-PAR reciprocity does indeed constitute an unambiguous measure of valence, it would be a welcome addition to the tool box of affective neuroscience.

REFERENCES


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