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Multi-level processing of emotions in life motion signals revealed through pupil responses

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Abstract

Perceiving emotions from the movements of other biological entities is critical for human survival and interpersonal interactions. Here, we report that emotional information conveyed by point-light biological motion (BM) triggered automatic physiological responses as reflected in pupil size. Specifically, happy BM evoked larger pupil size than neutral and sad BM, while sad BM induced a smaller pupil response than neutral BM. Moreover, this happy over sad pupil dilation effect is negatively correlated with individual autistic traits. Notably, emotional BM with only local motion features retained could also exert modulations on pupils. Compared with intact BM, both happy and sad local BM evoked stronger pupil responses than neutral local BM starting from an earlier timepoint, with no difference between the happy and sad conditions. These results revealed a fine-grained pupil-related emotional modulation induced by intact BM and a coarse but rapid modulation by local BM, demonstrating multi-level processing of emotions in life motion signals. Taken together, our findings shed new light on BM emotion processing, and highlight the potential of utilizing the emotion-modulated pupil response to facilitate the diagnosis of social cognitive disorders.

eLife Assessment

This **important** study provides **convincing** evidence that emotional information in biological motion can induce different patterns of pupil responses, which could serve as a behavioral marker of an autistic trait. These results broaden our understanding of how emotional biological motion can automatically trigger physiological changes and reveal the potential of using emotional-modulated pupil response to facilitate the diagnosis of social cognitive disorders. The work will be of broad interest to cognitive neuroscience, psychology, affective neuroscience, and vision science.

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Introduction

Perceiving and interpreting emotions from various social signals is critical for human social functioning, which enables us to infer the intentions of our conspecifics and further facilitates interpersonal interactions. Facial expressions present the most common non-verbal social communicative signals regarding others' affective states and intentions (C. Frith, 2009). In addition to faces, the movement of biological organisms serves as another essential type of social signal carrying significant emotional information, and this information remained salient even from a far distance (de Gelder, 2006). The human visual system is highly sensitive to such signals that we can readily decipher emotions from biological motion (BM), even when it was portrayed by several point lights attached to the major joints (Johansson, 1973; Troje, 2008). Moreover, it has been found that happy point-light walkers were recognized faster and more accurately than sad, angry or neutral walkers, demonstrating a happiness superiority (Lee & Kim, 2016; Spencer et al., 2016). While these studies provided some insights into the emotion processing mechanism of BM, they relied mainly on the explicit identification and active evaluation of BM emotions. Importantly, the encoding of emotional information also involves a rather automatic and implicit process that is independent of the participant's explicit identifications (Critchley et al., 2000; Lange et al., 2003; Okon-Singer et al., 2013; Shafer et al., 2012). Notably, this implicit aspect of emotion processing could be even more effective in probing individual differences and social deficits (Kana et al., 2015; Keifer et al., 2020; Kovarski et al., 2018; Wong et al., 2008), as it requires the intuitive processing of emotions that could not be learned (U. Frith, 2004). For example, research has shown that individuals with autistic disorders showed altered neural activities during implicit but not explicit emotion processing of natural scenes (Kana et al., 2015). These observations highlighted the importance of using objective measurements to investigate the implicit and automatic aspect of BM emotion processing.

The pupil response hence serves as a promising approach for reliably unfolding the implicit emotion processing of BM as it adds no extra task requirements to the cognitive process (Burley et al., 2017). In particular, pupil size is related to the activity of the automatic nervous system mediated by the locus coeruleus norepinephrine (LC-NE), which not only responds to physical light, but also reflects the underlying cognitive state (Joshi & Gold, 2019). Moreover, it could spontaneously capture the current subjective state and is thus useful for revealing the time course of the related cognitive processing (de Gee et al., 2014; Graves et al., 2021; Kloosterman et al., 2015; Oliva & Anikin, 2018). Recently, this measurement has been introduced to the field of emotion perception, and emerging evidence has suggested that emotions conveyed by social signals (e.g., faces, voices) could modulate pupil responses. For instance, happy and angry dynamic faces elicited a pupil dilation effect as compared with sad and neutral faces (Burley & Daughters, 2020; Prunty et al., 2021). Noticeably, the point-light BM, as another critical type of social signal, also conveyed salient affective information. Besides, its emotion processing mechanism is closely connected with that of faces (Alaerts et al., 2011; Becker et al., 2011; Lee & Kim, 2016; Yuan et al., 2024). However, it remains unequivocal whether the pupil also responds to the emotional information carried by the minimalistic point-light walker display. Such physiological observation can faithfully uncover the implicit emotion processing of BM, and it also expands the existing line of inquiry on the emotion processing of social cues.

To fill this gap, the current study implemented the pupil recording technique together with the passive viewing paradigm to explore the automatic and implicit emotion processing of BM. We first investigated the pupil responses to point-light walkers with different emotions (i.e., happy, sad, and neutral). In addition to intact emotional BM sequences, we also tested scrambled emotional BM sequences, which lack the gestalt of a global figure but preserve the same local motion components as the intact walkers. Recent studies have shown that such local motion

signals play an essential role in conveying biologically salient information such as animacy, and walking direction (Chang & Troje, 2008, 2009; Troje & Westhoff, 2006). This study went further to investigate whether this local BM signal could convey emotional information and elicit corresponding pupil responses. Moreover, given that emotion perception from social signals is generally impaired in individuals with autism (Harms et al., 2010; Hubert et al., 2006), we also took the individual autistic traits into consideration by measuring the autistic tendencies in normal populations with the Autistic Quotient (AQ) questionnaire (Baron-Cohen et al., 2001).

Results

Experiment 1a-b: Intact emotional BM

In Experiment 1a, we investigated whether emotional BM could automatically exert influences on pupil responses. Specifically, participants were instructed to passively view the intact happy/sad/neutral BM with their pupil responses being recorded simultaneously (see **Fig.1**). A cluster-based permutation analysis was applied to illustrate the time course of emotional modulations on pupil responses. Results showed that the happy BM induced a significant pupil dilation effect than the neutral BM from 1750 ms to 3200 ms (see **Fig.2A**). Conversely, the sad BM evoked a significantly smaller pupil response than the neutral BM, which starts from 1850 ms until the end of the stimulus presentation (see **Fig. 2A**). Moreover, the happy BM evoked a significantly larger pupil response as compared to the sad BM in a longstanding time window, ranging from 1200 ms until the disappearance of the display (see **Fig.2A**).

We then computed the mean pupil size by collapsing the pupillometry across all time points, ranging from the onset of the stimuli to the end of the stimuli presentation. A one-way repeated measures ANOVA was conducted on the mean pupil size for each emotional condition (happy, sad, neutral), and the results showed a significant main effect of emotional condition ($F(1.4, 32.2) = 8.92$, $p = .002$, $\eta_p^2 = 0.28$; Greenhouse-Geisser corrected, **Fig.3A**). On average, the happy BM induced a significantly larger pupil response than the neutral BM ($t(23) = 2.73$ $p = .024$, Cohen's $d = 0.56$, 95% confidence interval (CI) for the mean difference = [0.04, 0.27]; Holm-corrected, $p = .036$ after Bonferroni correction, **Fig.3A**). In contrast, the sad BM evoked a significantly smaller pupil size than the neutral BM (sad vs. neutral: $t(23) = -2.43$, $p = .024$, Cohen's $d = 0.50$, 95% CI for the mean difference = [-0.35, -0.03]; Holm-corrected, $p = .071$ after Bonferroni correction, **Fig.3A**). Moreover, the happy BM evoked a significantly larger pupil size than the sad BM ($t(23) = 3.34$, $p = .009$, Cohen's $d = 0.68$, 95% CI for the mean difference = [0.13, 0.55]; Holm-corrected, $p = .009$ after Bonferroni correction, **Fig.3A**), which echoes with former studies showing a happiness advantage in BM processing (Actis-Grosso et al., 2015; Lee & Kim, 2016; Spencer et al., 2016; Yuan et al., 2023). Importantly, the observed happy over sad dilation effect is negatively correlated with the individual autistic traits ($r(23) = -0.47$, $p = .022$, 95% CI for the mean difference = [-0.73, -0.08], **Fig. 4A**), indicating a compromised ability to perceive emotions from BM among individuals with higher autism tendencies. No significant correlations were found between AQ and other pupil modulation effects (**Fig. 4B-C**). Additionally, no significant correlations were observed between AQ and the original pupil responses induced by happy/neutral/sad BM (see Supplementary Material for more details).

To strengthen the correlation results, we conducted a replication experiment (Experiment 1b) and added a test-retest examination to further assess the reliability of our measurements. Specifically, a new group of participants were recruited to perform the identical task, and they were asked to return to the lab for a retest. The results again revealed the main effect of emotional condition in both the first test ($F(2, 46) = 12.0$, p .001, $\eta_p^2 = 0.34$, **Fig.3B**) and the second test ($F(2, 46) = 14.8$, $p < .001$, $\eta_p^2 = 0.39$, **Fig.3C**). The happy BM induced a significantly larger pupil response than the neutral BM (First Test: $t(23) = 2.60$, $p = .022$, Cohen's $d = 0.53$, 95% CI for the mean difference = [0.02, 0.14], Holm-corrected, $p = .048$ after Bonferroni correction, **Fig.3B**; Second Test: $t(23) =$

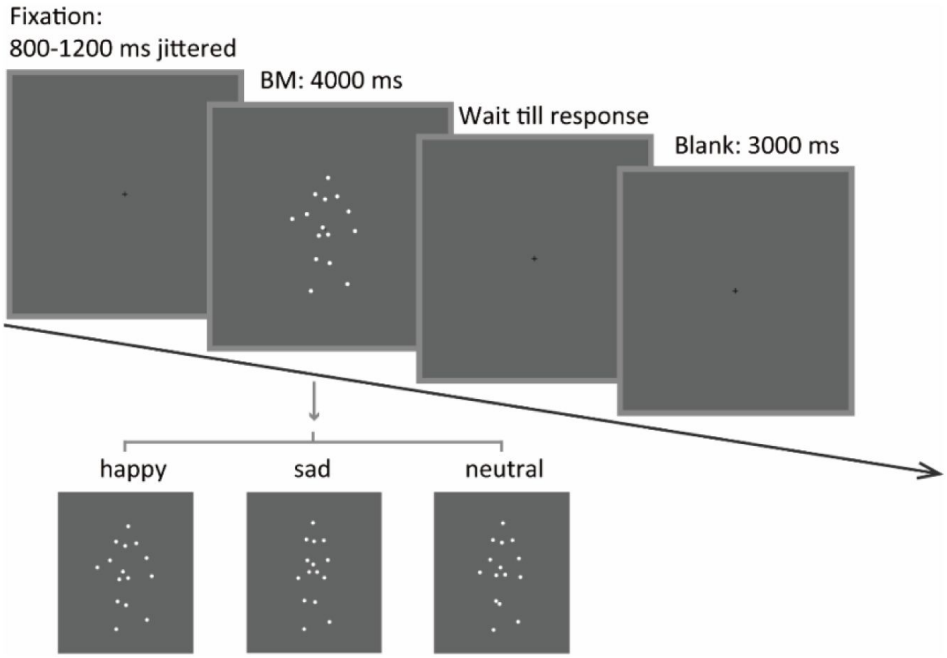

Fig. 1.

Schematic representation of the experimental procedure and the stimuli in Experiment 1a. A happy/sad/neutral BM walker turning 45 degrees leftwards or rightwards was presented at the center of the screen for 4000 ms. Participants were instructed to maintain their fixation on the BM stimuli during stimulus presentation and to continue the procedure through key pressing.

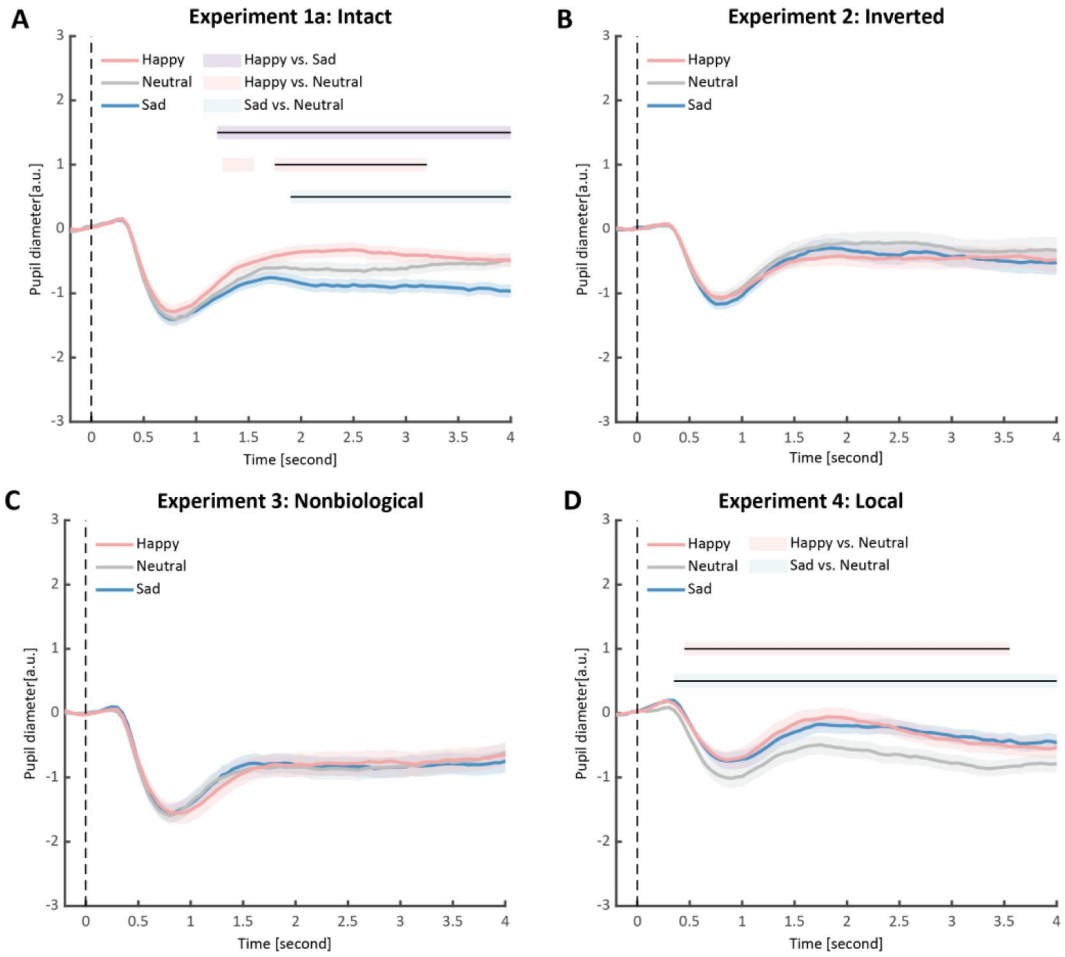

Fig. 2.

Time course of pupil responses to happy, sad, and neutral BM in Experiments 1-4. Solid lines represent pupil diameter under each emotional condition as a function of time (happy: red; sad: blue; neutral: gray); shaded areas represent the SEM between participants; colored horizontal lines indicate periods during which there are statistically significant differences among conditions at p <0.05; and black horizontal lines indicate significant differences after cluster-based permutation correction. All the pupil data are in arbitrary units (a.u.) (A) In Experiment 1a, the happy BM evoked larger pupil response as compared to the sad and neutral BM, and the sad BM evoked smaller pupil size than the neutral BM. (B) In Experiment 2, the inverted BM failed to produce such emotional modulation effects. (C) In Experiment 3, the emotional BM that is deprived of the local motion feature exerted no emotional modulation on pupil responses. (D) In Experiment 4, both the happy and sad local BM induced a larger pupil response than the neutral local BM, and such dilation effect started from a relatively early time point.

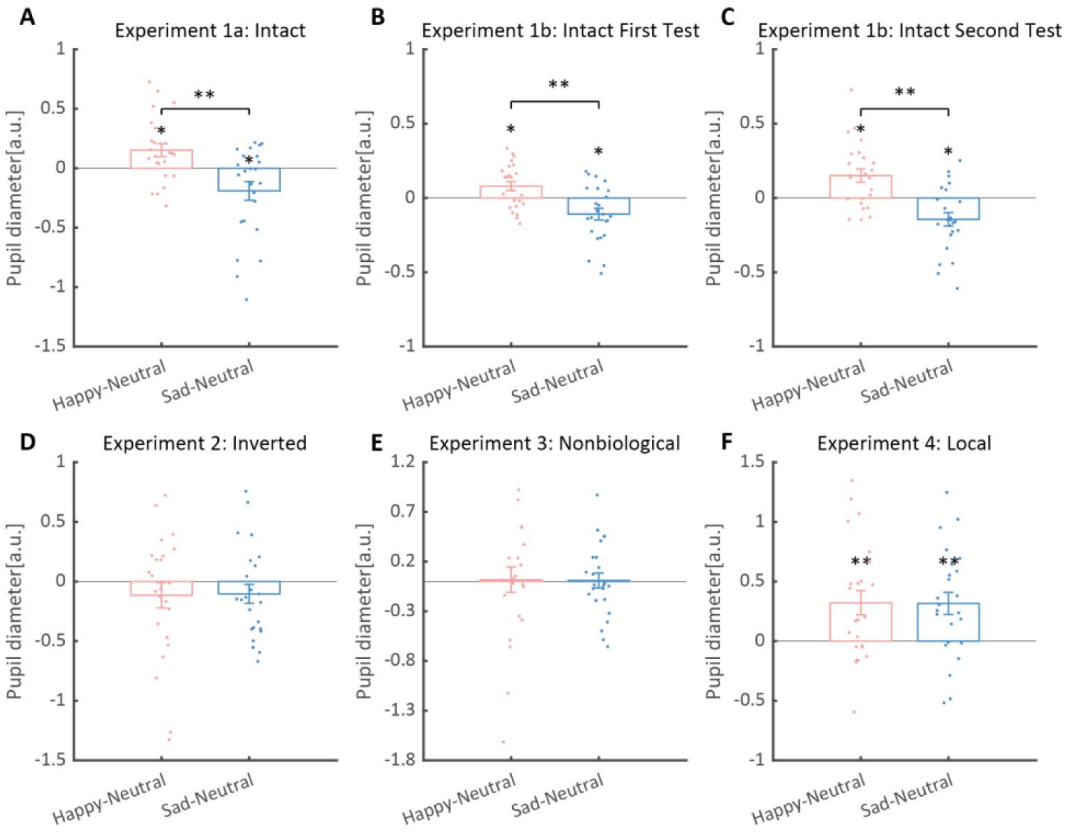

Fig. 3.

Normalized mean pupil responses using the neutral condition as baseline, plotted against happy and sad conditions, and the relevant correlation. (A) In Experiment 1a, the group average pupil response to happy intact BM is significantly larger than that to sad and neutral BM, while the pupil size induced by sad BM is significantly smaller than that evoked by neutral BM. (B-C) Moreover, such an emotional modulation on pupil sizes was again identified in the test and retest of the replication experiment (Experiment 1b). (D) In Experiment 2, no significant differences in pupil responses were observed for inverted BM. (E) In Experiment 3, when the biological characteristic was deprived from the emotional BM, it failed to induce any modulations on pupil sizes. (F) In Experiment 4, both the happy and sad local BM induced a significantly larger pupil size than neutral local BM, with no significant difference between the happy and sad condition. All the pupil data are in arbitrary units (a.u.). Each point represents one individual data. Error bars showed standard errors of the mean. * $p < .05$, ** $p < .01$.

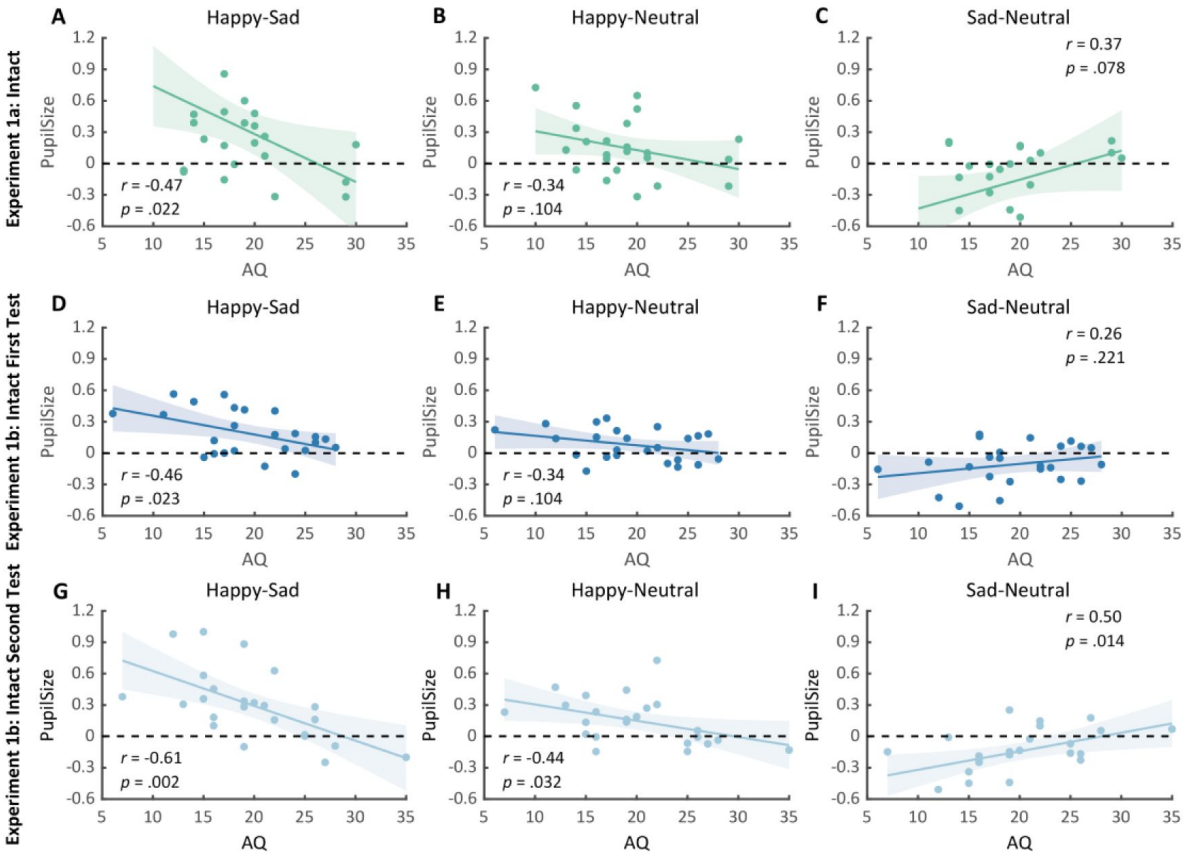

Fig. 4.

Correlation results for pupil modulations and AQ scores in Experiment 1a and its replication experiment (Experiment 1b). (A) In Experiment 1a, a significant negative correlation was found between the happy over sad pupil dilation effect and individual autistic quotient (AQ). (B-C) No other significant correlations were found. (D-F) The first test of Experiment 1b replicated the negative correlation between happy over sad pupil dilation effect and AQ. Similarly, no other significant correlations were found. (G) In the second test, the negative correlation between happy over sad pupil dilation effect and AQ was similarly observed and even stronger. (H-I) Moreover, we've additionally observed a significant positive correlation between AQ and the happy minus neutral pupil dilation effect, and a significant negative correlation between AQ and the sad minus neutral pupil constriction effect.

3.36, p = .005, Cohen's d = 0.68, 95% CI for the mean difference = [0.06, 0.24], Holm-corrected, p = .008 after Bonferroni correction, **Fig.3C**). On the contrary, the sad BM induced a significantly smaller pupil response than the neutral BM (First Test: $t(23)$ = -2.77, p = .022, Cohen's d = 0.57, 95% CI for the mean difference = [-0.19, -0.03], Holm-corrected, p = .033 after Bonferroni correction, **Fig.3B**; Second Test: $t(23)$ = -3.19, p = .005, Cohen's d = 0.65, 95% CI for the mean difference = [-0.24, -0.05], Holm-corrected, p = .012 after Bonferroni correction, **Fig.3C**). Besides, the happy BM induced significantly larger pupil response than the sad BM (First Test: $t(23)$ = 4.23, p < .001, Cohen's d = 0.86, 95% CI for the mean difference = [0.10, 0.28], Holm-corrected, p < .001 after Bonferroni correction, **Fig.3B**; Second Test: $t(23)$ = 4.26, p < .001, Cohen's d = 0.87, 95% CI for the mean difference = [0.15, 0.44], Holm-corrected, p < .001 after Bonferroni correction, **Fig.3C**). The results of the cluster-based permutation analysis were also similar (see Supplementary Material for more details).

Notably, we successfully replicated the negative correlation between the happy over sad dilation effect and individual autistic traits ($r(23)$ = -0.46, p = .023, 95% CI for the mean difference = [-0.73, -0.07], **Fig.4D**) in the first test of Experiment 1b. No other significant correlations were found (see **Fig.4E-F**). Moreover, in the second test, such a correlation was similarly found and was even stronger ($r(23)$ = -0.61, p = .002, 95% CI for the mean difference = [-0.81, -0.27], **Fig.4G**). A test-retest reliability analysis was performed on the happy over sad pupil dilation effect and the AQ score, and the results showed robust correlations (r(happy-sad pupil size)= 0.56; r(AQ)= 0.90) and strong test-retest reliabilities (α(happy-sad pupil size)= 0.60; α(AQ)= 0.82). Furthermore, in the second test, we've additionally observed a significant negative correlation between AQ and the happy minus neutral pupil dilation effect ($r(23)$ = -0.44, p = .032, 95% CI for the mean difference = [-0.72, -0.04], **Fig.4H**), and a significant positive correlation between the sad minus neutral pupil size and AQ ($r(23)$ = 0.50, p = .014, 95% CI for the mean difference = [0.12, 0.75], **Fig.4I**). This indicated that the overall correlation between happy over sad dilation effect and AQ was the aggregate outcome of the diminished pupil modulations by happy and sad BM for high AQ individuals.

Overall, these results demonstrated a robust and replicable modulation of emotions embedded in the minimized point-light walker on pupil sizes: the happy BM evoked a larger pupil response than the neutral BM, while the sad BM evoked a smaller pupil size as compared to the neutral one. Importantly, a significant negative correlation between AQ and happy over sad pupil dilation effect was consistently found, and such effect was caused by a general attenuation in BM emotion perception sensitivity among individuals with high autistic tendencies.

Experiment 2: Inverted emotional BM

To rule out the possibility that the difference in low-level visual features rather than the emotional information per se might account for the obtained emotional modulation effect, we presented observers with the inverted BM stimuli that shared the exact perceptual features with their upright counterparts in Experiment 2. The cluster-based permutation analysis observed no significantly different time points among three emotional conditions (see **Fig. 2B**). An identical one-way repeated measures ANOVA was conducted, while no significant main effect of emotional condition on pupil responses was observed in inverted BM ($F(2, 46)$ = 0.95, p = .396, η_p^2 = 0.04, **Fig. 3D**). Besides, no significant correlations between AQ and pupil modulations were found (AQ with happy-sad pupil size: $r(23)$ = 0.14, p = .512, 95% CI for the mean difference = [-0.28, 0.52]; AQ with happy-neutral pupil size: $r(23)$ = 0.26, p = .227, 95% CI for the mean difference = [-0.16, 0.60]; AQ with sad-neutral pupil size: $r(23)$ = 0.19, p = .376, 95% CI for the mean difference = [-0.23, 0.55]). Critically, the mixed 2 (orientation: upright, inverted) × 3 (emotional condition: happy, sad, neutral) ANOVA on the average pupil size obtained in Experiment 1a and Experiment 2 showed a significant interaction between orientation and emotional condition ($F(2, 92)$ = 4.38, p = .013, η_p^2 = 0.09), which could be attributed to the diminishment of emotional modulation in inverted BM. This indicated that the observed emotional modulation of pupil responses did not arise from low-level visual differences.

Experiment 3: Nonbiological Motion

Given the ample evidence showing that local motion feature is essential for the perception of biologically significant information from BM (Chang & Troje, 2008, 2009; Troje & Westhoff, 2006; L. Wang & Jiang, 2012), we moved forward to explore the role of local motion feature in the emotion processing of BM. In Experiment 3, we presented observers with non-BM stimuli, which were derived from the fragments identical to emotional BM but with critical local characteristics removed (Chang & Troje, 2009). The permutation analysis found no significantly different time points in pupil responses to the happy, sad, and neutral conditions (see **Fig. 2D**). Results of the one-way repeated ANOVA on the average pupil sizes also showed no significant main effect of emotional condition ($F(1.6, 35.7) = 0.02$, $p = .964$, $\eta_p^2 = 0.00$; Greenhouse-Geisser corrected, see **Fig. 3C**). Similarly, no pupil modulation effects significantly correlated with AQ (AQ with happy-sad pupil size: $r(23) = -0.03$, $p = .896$, 95% CI for the mean difference = [-0.43, 0.38]; AQ with happy-neutral pupil size: $r(23) = 0.01$, $p = .956$, 95% CI for the mean difference = [-0.39, 0.41]; AQ with sad-neutral pupil size: $r(23) = 0.06$, $p = .777$, 95% CI for the mean difference = [-0.35, 0.45]). Moreover, combining the results obtained from Experiment 1a and Experiment 3, we found a significant interaction between stimulus type (intact, nonbiological) and emotional condition (happy, sad, neutral) ($F(2, 92) = 3.22$, $p = .045$, $\eta_p^2 = 0.07$), which was caused by the lack of emotional modulation in nonbiological stimuli. These findings together showed that the removal of local characteristics greatly disrupted the emotional modulations on observers' pupil responses, providing conceivable evidence for the critical contribution of local motion features in emotion perception from the BM signal.

Experiment 4: Local emotional BM

In Experiment 4, we went further to examine whether local BM alone could carry emotional information and exert a similar modulation effect on pupil size. In particular, we adopted the well-established local BM stimuli, the scrambled BM, whose local motion feature was retained while the global configuration information was completely disrupted. Both the explicit and implicit emotion processing of the scrambled BM were investigated to provide a thorough view of emotion perception from local BM. Specifically, a group of observers viewed the scrambled BM and made explicit behavioral judgments on the emotional information contained in the scrambled BM. The results showed that observers could successfully recognize the emotions contained in scrambled BMs with the average accuracy reaching significantly above 50% (M ± SD = 83% ± 2.8%, $p < .001$). This indicated that scrambled BM conveyed recognizable emotional information.

Furthermore, we investigated the pupil responses to scrambled happy, sad, and neutral BMs to explore the automatic and implicit processing of local emotional BM. The consecutive cluster-based permutation analysis further showed that both the scrambled happy and sad BM evoked larger pupil sizes than the scrambled neutral BM. Importantly, such effect appeared in a rather early time window and could last for a quite long time (happy vs. neutral: 450 ms -3550 ms; sad vs. neutral: 350 ms-4000 ms, see **Fig. 2D**). The one-way repeated measures ANOVA on the mean pupil size indicated a significant main effect of emotional condition ($F(2, 46) = 6.47$, $p = .003$, $\eta_p^2 = 0.22$, **Fig. 3F**). Follow-up analysis showed that the scrambled happy BM induced a significantly larger pupil response than the scrambled neutral BM ($t(23) = 3.22$, $p = .008$, Cohen's $d = 0.66$, 95% CI for the mean difference = [0.12, 0.53]; Holm-corrected, $p = .011$ after Bonferroni correction, **Fig. 3F**), which is similar to that observed in Experiment 1a-b. Notably, the scrambled sad BM also induced a larger pupil size than the neutral one ($t(23) = 3.41$, $p = .007$, Cohen's $d = 0.70$, 95% CI for the mean difference = [0.12, 0.53]; Holm-corrected, $p = .007$ after Bonferroni correction, **Fig. 3F**). Moreover, no difference in pupil size is observed between the happy and sad conditions ($t(23) = -0.07$, $p = .948$, Cohen's $d = 0.01$, 95% CI for the mean difference = [-0.23, 0.24]; Holm-corrected, $p = 1.000$ after Bonferroni correction, **Fig. 3F**). The observed effect could not be accounted for by perceptual differences (e.g., speed), as the happy and sad scrambled BM differed the most in low-level features, whereas they induced similar dilation effects on pupil sizes. Again, no significant

correlations were observed between AQ and pupil modulation effects (AQ with happy-sad pupil size: $r(23) = -0.21$, $p = .32$, 95% CI for the mean difference = [-0.57, 0.21]; AQ with happy-neutral pupil size: $r(23) = -0.13$, $p = .56$, 95% CI for the mean difference = [-0.50, 0.29]; AQ with sad-neutral pupil size: $r(23) = 0.13$, $p = .56$, 95% CI for the mean difference = [-0.29, 0.50]). Importantly, a mixed 2 (BM type: intact, local) × 3 (emotional condition: happy, sad, neutral) ANOVA on average pupil size was conducted to examine whether or not this emotional modulation effect of local BM varied from that of intact BM reported in Experiment 1a-b. Results revealed a significant interaction between BM type and emotional condition ($F(2, 92) = 7.76$, $p < .001$, $\eta_p^2 = 0.14$), indicating that emotions in intact and local BM exerted differential modulations on pupil size. This interaction is primarily driven by the vanishment of pupil modulation between happy and sad local BM. Overall, these findings showed that the local BM could convey significant emotional information, which could induce a pupil dilation effect regardless of its exact type. As compared to the intact BM, the emotional modulation observed in local BM is different because it occurs faster and is rather coarse.

Discussion

Life motion signals convey salient emotional information that is crucial for human survival and social interactions (Johansson, 1973; Troje, 2008). Here, we reported that such emotional clues carried by the point-light BM walker could exert a modulation effect on pupil responses. Specifically, the happy BM significantly dilated pupils as compared to the neutral BM, and the sad BM evoked smaller pupil responses than the neutral BM, showing distinct emotional modulations on pupil responses that depend on the emotional contents. Moreover, this emotional modulation of pupil responses could not be explained by the low-level differences, as viewing inverted BMs failed to induce such effects. Importantly, when the emotional BM was deprived of the local motion feature through the removal of accelerations, it failed to induce any modulation on pupil responses. Furthermore, the scrambled emotional BM with only the local motion feature retained could still produce a modulation effect on pupil size. Noticeably, this modulation is rapid but rather coarse: viewing the scrambled happy and sad BM would evoke greater pupil size than the scrambled neutral BM in a relatively early time window, while no significant difference was observed between the scrambled happy and sad BM. Taken together, these findings revealed multi-level processing of emotions in life motion signals: the global emotional BM modulated pupil size in a fine-grained manner that discriminates each type of emotion, and the local emotional BM exerted a coarse but rapid modulation on pupil size.

The emotion processing of BM has been investigated in former studies using explicit behavioral detection and recognition paradigm. It has been reported that happy BM is more rapidly identified and detected as compared to sad and neutral BM (Actis-Grosso et al., 2015; Lee & Kim, 2016; Spencer et al., 2016), while other research observed no such superiority (Chouchourelou et al., 2006). Here, the present study adopted the novel and objective pupillometry index to investigate the emotion processing of BM from a physiological aspect. The pupil index, as a direct reflection of individual arousal level and cognitive state, served as a powerful tool for faithfully and automatically reflecting the implicit processing of emotions in BM. Previous studies have reported larger pupil responses towards emotional images as compared to neutral images, with no significant differences observed between the positive and negative conditions (Bradley & Lang, 2015; Bradley et al., 2008; Snowden et al., 2016). However, these studies mostly adopted complex emotional scene images that conveyed rather general emotional information. When it comes to the specific emotion cues (e.g., happy, sad) delivered by our conspecifics through biologically salient signals (e.g., faces, gestures, voices), the results became intermixed. Some studies reported pupil dilatory effects towards fearful, disgusted, angry, sad faces but not happy faces (Burley et al., 2017). On the contrary, other studies observed larger pupil responses for happy as compared to sad, fearful, and surprised faces (Aktar et al., 2018; Burley & Daughters, 2020; Jessen et al., 2016; Prunty et al., 2021). These conflicting results could be due to the

low-level confounds of emotional faces (e.g., eye size) (Carsten et al., 2019; Harrison et al., 2006). Similar to faces, BM also conveyed salient clues concerning the emotional states of our interactive partners. However, they were highly simplified, and deprived of various irrelevant visual confounders (e.g., body shape). Here, we reported that the happy BM induced a stronger pupil response than the neutral and sad BM, lending support to the happy dilation effect observed with faces (Burley & Daughters, 2020; Prunty et al., 2021). Moreover, it helps ameliorate the concern regarding the low-level confounding factors by identifying similar pupil modulations in another type of social signal with distinctive perceptual features.

It has long been documented that pupil size reflects the degree of cognitive effort and attention input (Joshi & Gold, 2019; van der Wel & van Steenbergen, 2018), and indexes the noradrenalin activity in emotion processing structures like amygdala (Dal Monte et al., 2015; Harrison et al., 2006; Liddell et al., 2005). Thus, the happy dilation effect suggests that the happy BM may be more effective in capturing attention and evoking emotional arousal than the neutral and sad BM, which echoes with the happiness superiority reported in the explicit processing of BM (Lee & Kim, 2016; Spencer et al., 2016; Yuan et al., 2023). Instead, the sad constriction effect potentially indicates a disadvantage for sad BM in attracting visual attention and evoking emotional arousal than neutral BM. In line with this, it has been found that infants looked more at the happy BM walker when displayed in pair with the neutral walker, whereas they attended less to the sad walker as compared to the neutral one (Ogren et al., 2019). Besides, it has been revealed by neural studies that, the happy emotion evoked stronger activities in emotionally relevant brain regions including the amygdala, the extrastriate body area (EBA), and the fusiform body area (FBA), while the sad emotion failed to induce such effects (Peelen et al., 2007; Ross et al., 2019). Still, future studies are needed to provide further evidence regarding the happy advantage and the sad disadvantage in attracting visual attention. For example, in addition to pupil size, the microsaccades also provided valuable insights into attention processes (Baumeler et al., 2020; Engbert & Kliegl, 2003; Meyberg et al., 2017), and potentially involved shared neural circuits with pupil responses (Hafed et al., 2009; Hafed & Krauzlis, 2012; C.-A. Wang et al., 2012). Future studies could combine the microsaccade index with pupil size to provide a more thorough understanding of BM emotion processing.

Notably, the happy over sad dilation effect was negatively correlated with autistic traits: individuals with greater autistic tendencies showed decreased sensitivities to emotions in BM. In fact, the perception of biological motion (BM) has long been considered an important hallmark of social cognition, and abundant studies reported that individuals with social cognitive deficits (e.g., ASD) were impaired in BM perception (Blake et al., 2003; Freitag et al., 2008; Klin et al., 2009; Nackaerts et al., 2012). More recently, it has been pointed out that the extraction of more complex social information (e.g., emotions, intentions) from BM, as compared to basic BM recognitions, could be more effective in detecting ASDs (Federici et al., 2020; Koldewyn et al., 2009; Parron et al., 2008; Todorova et al., 2019). Specifically, a meta-analysis found that the effect size expanded nearly twice when the task required emotion recognition as compared to simple perception/detection (Todorova et al., 2019). However, for the high-functioning ASD individuals, it has been reported that they showed comparable performance with the control group in explicitly labelling BM emotions, while their responses were rather delayed (Mazzoni et al., 2021). This suggests that ASD individuals could adopt compensatory strategies to complete the explicit BM labelling task, while their automatic responses remained impaired. Such an observation highlights the importance of using more objective measures that do not rely on subjective reports to investigate the intrinsic perception of emotions from BM and its relationship with ASD-related social deficits. The current study thus introduced pupil size measurement to this field, and we combined it with the passive viewing task to investigate the more automatic aspect of BM emotion processing. In addition to diagnostic ASDs, the non-clinical general population also manifested autistic tendencies that followed normal distribution and demonstrated substantial heritability (Hoekstra et al., 2007). Here, we focused on the autistic tendencies in the general population, and our results showed that the automatic emotion processing of BM stimuli was

impaired in individuals with high autistic tendencies, lending support to previous studies (Hubert et al., 2006; Nackaerts et al., 2012; Parron et al., 2008). The more detailed test-retest examination further confirmed such a correlation and illustrated a general diminishment in BM emotion perception ability (happy and sad) for high AQ individuals. Given that pupil measurement does not require any explicit verbal reports, it is easily attainable in children and even infants. Thus, the pupil modulation effect obtained here may serve as a sensitive and reliable physiological marker for detecting early social cognitive disorders in both clinical and non-clinical populations.

Remarkably, our findings highlighted the central role of local motion feature in modulating pupil responses towards emotional information contained in BM. Previous studies have shown that human visual system is highly sensitive to the local BM stimulus, whose global configural information is completely deprived through spatially scrambling (Troje & Westhoff, 2006). For example, it has been demonstrated that scrambled BM could perform as intact BM in lengthening subjective temporal perception (L. Wang & Jiang, 2012). Besides, such local motion feature also served as a basic pre-attentive feature in visual search (L. Wang et al., 2010) and could further induce a significant reflexive attentional effect (L. Wang et al., 2014; Yu et al., 2020). Moreover, the deprivation of the local BM feature would greatly disrupt the perception of the contained biologically salient information, such as animacy and walking direction (Chang & Troje, 2008, 2009; Yu et al., 2020). Here, we extended this line of research to the emotional domain by demonstrating that the local BM component is also critical for the processing of emotional information: when the local BM feature is disrupted, the modulation of emotions on pupil size completely disappears. Furthermore, the emotional BM that retained only local motion features could still exert a salient modulation effect on pupil size. In particular, the happy and sad local BM induced a significant pupil dilation effect as compared to the neutral local BM stimuli. Intriguingly, this dilation effect is independent of the specific emotion category but reflects a general activation caused by the affective salient information. In addition, this non-selective pupil dilation effect in local BM appeared in a relatively early time window, indicating that the extraction of emotions from local BM is rapid. Taken together, these findings identified a coarse but rapid emotion processing mechanism in local BM that could promptly detect the emotional information therein without the aid of the global shape.

Moreover, our findings revealed the existence of distinct emotional modulations in intact and local BM, respectively, as evidenced by variations in pupil responses. In particular, the happy intact BM induced a significant pupil dilation effect as compared to the neutral one, whereas the sad intact BM led to pupil constriction. Interestingly, both the happy and sad local BM resulted in pupil dilation effects relative to the neutral local BM, and such effect occurred at a relatively early time point. This distinctive pupil modulation effects observed in intact and local BM could be elucidated by a multi-level emotion processing mechanism. Specifically, even though both the intact and local BM conveyed important life information, the local BM is deprived of the global configural information (Chang & Troje, 2008, 2009; Simion et al., 2008). Thus, its emotion processing potentially occurred at a more basic and preliminary level, responding to the general affective salient information without engaging in further detailed analysis. Importantly, similar dissociated emotion processing phenomenon has been observed in another important type of emotional signal with analogous function (i.e., facial expression). For example, happy and fearful faces induced differential amygdala activations when perceived consciously, yet they induced comparable amygdala activations when suppressed (Williams et al., 2004). Moreover, it has been argued that there exist two parallel pathways for facial expression processing: a slow route that conveys fine-grained facial features along cortical areas to the amygdala, and a rapid subcortical pathway that directly transfers emotional information to amygdala without detailed analysis, which is known as the “quick and dirty” route (Garrido et al., 2012; Johnson, 2005; Méndez-Bértolo et al., 2016; Vuilleumier et al., 2003). It is probable that the emotion processing of the local and intact point-light BM potentially functions in a manner similar to that of the faces, with the former serving as a primary detection mechanism that automatically captures emotionally significant information conveyed by animate agents without detailed analysis, and the latter

aiding in more specific emotion identification based on fine-grained analyses of their motion pattern and global shape. Compatible with this view, recent studies have reported that the emotion processing of face and BM was very similar. For example, they both showed a happiness superiority in visual detection (Becker et al., 2011; Lee & Kim, 2016; Nackaerts et al., 2012) and guiding social attention (Yuan et al., 2023). Moreover, their emotion processing involved potentially overlapping brain regions (e.g., amygdala, STS) (Alaerts et al., 2014; Engell & Haxby, 2007; Peelen et al., 2007; Pessoa & Adolphs, 2010). Overall, our findings, together with the former evidence, suggest dissociated emotion processing for BM in the human brain akin to the dual-route model reported in faces, and further imply a general "emotional brain" that can be shared by different types of social signals. Still, future studies are needed to implement neuroimaging techniques to directly identify the brain regions involved in the emotion processing of local and global BM signals.

To conclude, the current study clearly demonstrated that the emotional information conveyed by point-light BM modulated pupil responses. The intact BM exerted a fine-grained emotional modulation on pupil sizes, while disrupting the contained local motion characteristic would deteriorate the observed modulations. Moreover, BM with only local motion features retained could exert a fast but rather coarse modulation on pupillometry. These findings together highlight the critical role of local motion feature in BM emotion processing, and further reveal the multi-level emotion processing of BM. Notably, the observed emotional modulation in intact BM is associated with individual autistic traits, suggesting the potential of utilizing the emotion-modulated pupil responses to facilitate the diagnosis of social cognitive disorders.

Methods

Participants

A total of 144 participants (52 males, 92 females) ranging from 18 to 30 years old (M ± SD = 23.0 ± 2.6) were recruited, with 24 in Experiment 1a, 24 in Experiment 1b, 24 in Experiment 2, 24 in Experiment 3, 24 in the behavioral part of Experiment 4, and 24 in the eye recoding part of Experiment 4. All of the participants had normal or corrected-to-normal vision and gave written informed consent in accordance with the procedure and protocols approved by the institutional review board of the Institute of Psychology, Chinese Academy of Sciences. They were all naïve to the purpose of the experiments. Prior power analyses (*F* tests, repeated measures, within factors) using G*Power (Version 3.1.9.4; (Faul et al., 2007)) indicated that a sample size of 24 participants would afford 80% power with alpha at .05 to detect a moderate main effect (f = 0.27). This sample size was comparable to previous studies with similar designs (Nakakoga et al., 2020).

Stimuli

Stimuli were displayed using MATLAB (Mathworks, Inc.) together with the Psychtoolbox extensions (Brainard, 1997; Pelli, 1997) on a 23-inch LED monitor (1920 × 1080 at 60 Hz) with gray background (RGB: 100, 100, 100). The biological motion (BM) stimuli were adopted from Troje (Troje, 2008). Each comprised 15 point-light dots depicting the motions of the head, pelvis, thorax and major joints (i.e., shoulders, elbows, wrists, hips, knees, and ankles). Its emotional state was indexed by a normalized Z score on an axis that reflects the differences between happy and sad walkers in terms of a linear classifier. The scores were computed within a 10-dimensional subspace spanned by the first 10 principal components based on a Fourier-based representation of observers' emotional ratings of 80 actual walkers (half male) (Troje, 2008). We adopted the neutral walker that scored 0 on the linear axis, together with the happy walker 6 SDs into the happy part of the axis and the sad walker 6 SDs into the sad part of the axis (see *https://www.biomotionlab.ca/html5-bml-walker/* for an interactive animation). Besides, we turned the BM walkers 45 degrees leftwards or rightwards to maximize the visibility of expressive bodily cues (Roether et al., 2009). In Experiment 1a-1b, the upright emotional (happy, sad, or neutral) BM

walkers with two walking directions (45° leftwards or rightwards) were used. The velocity was 5.76 pixels/frame for the happy BM, 4.14 pixels/frame for the neutral BM, and 3.21 pixels/frame for the sad BM. This difference in velocity profile was considered an important signature for conveying emotional information, as the happy walker was characterized by a larger step pace and longer arm swing, and the sad walker would instead exhibit a slouching gait with short slow strides and smaller arm movement (Barliya et al., 2012; Chouchourelou et al., 2006; Halovic & Kroos, 2018; Roether et al., 2009). In Experiment 2, the stimuli were replaced with their inverted counterparts created by mirror flipping the upright BM vertically. In Experiment 3, we presented observers with the non-biological motion stimuli whose local BM characteristic was disrupted through acceleration removal. More specifically, each individual dot moved along the original path with a constant speed equal to the average speed of the dot (Chang & Troje, 2009). Such manipulation disrupted the local motion feature of the BM stimuli but kept the trajectories of individual point lights unchanged. In Experiment 4, observers viewed the scrambled BM stimuli, which were created by randomly relocating the point light dots within the region occupied by the original BM walker. In this manner, the local motion feature was preserved while the global configuration of the BM stimulus was entirely disrupted (Troje & Westhoff, 2006). Please refer to Supplementary Materials for the demonstrations displaying the motion stimuli used in Experiments 1-4.

Procedure

Participants were seated at a viewing distance of 60 cm to the computer screen with their heads on a chinrest to minimize their head movements. Their pupil size and eye position of the left eye were recorded using a video-based iView X Hi-Speed system (SMI, Berlin, Germany) at 500 Hz. In Experiment 1a, each trial began with a central fixation cross (0.2° × 0.2°) with variable duration (800-1200 ms), followed by an upright happy/sad/neutral point-light walker (half leftwards and half rightwards) presented centrally for 4000 ms. Participants were required to passively view the BM stimulus and continue the procedure by pressing the space bar. After that, a blank screen was displayed for 3000 ms (**Fig. 1**). There were 4 blocks of 30 trials, and participants were given a short break after every block. Besides, we also administered a 9-point calibration of the eye-tracker followed by a validation stage before each experimental block to ensure the data quality. Participants were told to maintain fixation on the center of the screen and not to blink during the stimuli presentation. Importantly, a replication experiment of Experiment 1a (Experiment 1b) was conducted, which followed the same protocol, with the change being that participants were asked to return to the lab for a retest after at least seven days. Experiment 2 is identical to Experiment 1a, except that the BM stimuli were changed to their inverted counterparts. In Experiment 3, we instead presented participants with the non-biological motion displays whose local motion feature was deprived through acceleration removal. In Experiment 4, we adopted the scrambled emotional BM stimuli, and investigated both the explicit and the implicit emotion processing of scrambled BM. Note that a group of participants was recruited to perform emotional judgments on the scrambled BM in order to investigate whether the emotional information carried by local BM signals could be explicitly identified. Another group of participants was enrolled for the pupil recording experiment, which followed the identical experimental procedure of Experiments 1-3. At the end of all experiments, participants were required to complete the 50-point Autism-spectrum Questionnaire (AQ), which measured the degree of autistic traits in the normal population (Baron-Cohen et al., 2001).

Data Analysis

The raw pupil data for each trial was first screened to remove eye blinks (either replaced by linear interpolation or with this trial discarded). Then, trials with pupil size deviating ±3 SDs from the mean were excluded from further analysis. Finally, the pupil size data were down-sampled to 20 Hz and baseline-corrected for each trial by subtracting the mean pupil size during the 200 ms pre-stimulus period. We computed the average pupil size for each emotional condition (happy, sad, or neutral) obtained by collapsing the pupillometry across all time points. Besides, to depict the time

course of pupil responses towards emotional BM, we further conducted a consecutive paired-sample *t*-test across all time points comparing different emotional conditions. The cluster-based permutation analysis was applied to avoid potential problems brought about by multiple comparisons (Einhauser et al., 2008). In this analysis, the computed *t*-values neighboring in time that exceeded a threshold ($p < 0.05$) were defined as clusters, and then summed to produce a cluster mass. The cluster mass was compared with a null distribution, which was generated by 2000 random permutations of the pupil data from different conditions. If the cluster mass fell beyond 95% of the null distribution ($\alpha = 0.05$), it was deemed to be statistically significantly different (Einhauser et al., 2008). The pupil size was analyzed and reported in arbitrary units (a.u.) without transforming into the actual unit (mm), as the relative change of the pupil size was of main interest.

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Editors

Reviewer #1 (Public review):

Summary:

Tian et al. investigated the effects of emotional signals in biological motion on pupil responses. In this study, subjects were presented with point-light biological motion stimuli with happy, neutral, and sad emotions. Their pupil responses were recorded with an eye tracker. Throughout the study, emotion type (i.e., happy/sad/neutral) and BM stimulus type (intact/inverted/non-BM/local) were systematically manipulated. For intact BM stimuli, happy BM induced a larger pupil diameter than neutral BM, and neutral BM also induced a larger pupil diameter than sad BM. Importantly, the diameter difference between happy and sad BM correlated with the autistic trait of individuals. These effects disappeared for the inverted BM and non-BM stimuli. Interestingly, both happy and sad emotions show superiority in pupil diameter.

Strengths:

(1) The experimental conditions and results are very easy to understand.
(2) The writing and data presentation are clear.
(3) The methods are sound. I have no problems with the experimental design and results.

https://doi.org/10.7554/eLife.89873.2.sa3

Reviewer #2 (Public review):

Summary:

Through a serial of four experiments, Yuan, Wang and Jiang examined pupil size responses to emotion signals in point-light motion stimuli. Experiment 1 examined upright happy, sad and neutral point-light biological motion (BM) walkers. The happy BM induced a significantly larger pupil response than the neutral, whereas the sad BM evoked a significantly smaller pupil size than the neutral BM. Experiment 2 examined inverted BM walkers. Experiment 3 examined BM stimuli with acceleration removed. No significant effects of emotion were found in neither Experiment 2 nor Experiment 3. Experiment 4 examined scrambled BM stimuli, in which local motion features were preserved while the global configuration was disrupted. Interestingly, the scrambled happy and sad BM led to significant greater pupil size than the scrambled neutral BM at a relatively early time, while no significant difference

between the scrambled happy and sad BM was found. Thus, the authors argue that these results suggest multi-level processing of emotions in life motion signals.

Strengths:

The experiments were carefully designed and well-executed, with point-light stimuli that eliminate many potential confounding effects of low-level visual features such as luminance, contrast, and spatial frequency.

Overall, I think this is a well-written paper with solid experimental results that support the claim of the authors, i.e., the human visual system may process emotional information in biological motion at multiple levels. Given the key role of emotion processing in normal social cognition, the results will be of interest not only to basic scientists who study visual perception, but also to clinical researchers who work with patients of social cognitive disorders. In addition, this paper suggests that examining pupil size responses could be a very useful methodological tool to study brain mechanisms underlying emotion processing.

https://doi.org/10.7554/eLife.89873.2.sa2

Reviewer #3 (Public review):

Summary:
The overarching goal of the authors was to understand whether emotional information conveyed through point-light biological motion can trigger automatic physiological responses, as reflected in pupil size.

Strengths:
This manuscript has several noticeable strengths: it addresses an intriguing research question that fills that gap in existing literature, presents a clear and accurate presentation of the current literature, and conducts a series of experiments and control experiments with adequete sample size. Yet, it also entails several noticeable limitations - especially in the study design and statistical analyses.

Assessment of the revision:

The authors have done a thorough job revising the manuscript, effectively addressing all of my previous concerns.

https://doi.org/10.7554/eLife.89873.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***eLife assessment:***
>
> *This study presents an important finding on the implicit and automatic emotion perception from biological motion (BM). The evidence supporting the claims of the authors is solid, although inclusion of a larger number of samples and more evidence for the discrepancy between Intact and local emotional BMs would have strengthened the study. The work will be of broad interest to perceptual and cognitive neuroscience.*

We express our sincere gratitude for the positive and constructive evaluation of our manuscript. We have now included more participants and conducted a replication experiment to strengthen our results.

Reviewer #1 (Public Review):

Summary:

Tian et al. investigated the effects of emotional signals in biological motion on pupil responses. In this study, subjects were presented with point-light biological motion stimuli with happy, neutral, and sad emotions. Their pupil responses were recorded with an eye tracker. Throughout the study, emotion type (i.e., happy/sad/neutral) and BM stimulus type (intact/inverted/non-BM/local) were systematically manipulated. For intact BM stimuli, happy BM induced a larger pupil diameter than neutral BM, and neutral BM also induced a larger pupil diameter than sad BM. Importantly, the diameter difference between happy and sad BM correlated with the autistic trait of individuals. These effects disappeared for the inverted BM and non-BM stimuli. Interestingly, both happy and sad emotions show superiority in pupil diameter.

Strengths:

(1) The experimental conditions and results are very easy to understand.

(2) The writing and data presentation are clear.

(3) The methods are sound. I have no problems with the experimental design and results.

Weaknesses:

(1) My main concern is the interpretation of the intact and local condition results. The processing advantage of happy emotion is not surprising given a number of existing studies. However, the only difference here seems to be the smaller (or larger) pupil diameter for sad compared to neutral in the intact (or local, respectively) condition. The current form only reports this effect but lacks in-depth discussions and explanations as to why this is the case.

Thanks for pointing this out, our apology for not making this point clear. It has long been documented that pupil size reflects the degree of cognitive effort and attention input (Joshi & Gold, 2019; van der Wel & van Steenbergen, 2018), and indexes the noradrenalin activity in emotion processing structures like amygdala (Dal Monte et al., 2015; Harrison et al., 2006; Liddell et al., 2005). Accordingly, we proposed that the smaller pupil response observed under the sad condition as compared to the neutral condition is because the sad biological motion (BM) could be less efficient in attracting visual attention and evoking emotional arousal. In line with this, it has been found that infants looked more at the neutral point-light walker when displayed in pair with the sad walker (Ogren et al., 2019), suggesting that the sad BM is less effective in capturing visual attention than the neutral BM. Besides, neural studies have revealed that, compared with other emotions (anger, happiness, disgust, and fear), the processing of sad emotion failed to evoke heightened activities in any emotionally relevant brain regions including the amygdala, the extrastriate body area (EBA) and the fusiform body area (FBA) (Peelen et al., 2007)(Peelen et al., 2007). The current study echoed with these previous findings by demonstrating a disadvantage for intact sad BM in evoking pupil responses. Notably, different from the intact sad BM, the local sad BM would instead induce stronger pupil responses than the neutral local BM. This distinctive pupil modulation effect observed in intact and local sad BM could be explained as a multi-level emotion processing model of BM. Specifically, even though both the intact and local BM conveyed important life information (Chang & Troje, 2008, 2009; Simion et al., 2008), the latter is deprived of the global form feature. Hence, the processing of emotions in local BM may occur at a more basic and preliminary level, responding to the general affective salient emotion information (happy and sad) without detailed analysis. In fact, similar dissociated emotion processing phenomenon has been observed in another important type of emotional signal with

analogous function (i.e., facial expression). For example, happy and fearful faces elicited differential amygdala activations when perceived consciously. However, they elicited comparable amygdala activations when suppressed (Williams et al., 2004). Moreover, it has been proposed that there exist two parallel routes for facial expression processing: a quick but coarse subcortical route that detects affective salient information without detailed analysis, and a fine-grained but slow cortical route that discriminates the exact emotion type. Similarly, the dissociated emotion processing in local and intact BM may function in the same manner, with the former serving as a primary emotion detection mechanism and the latter serving as a detailed emotion discrimination mechanism. Still, future studies adopting more diverse experimental paradigms and neuroimaging techniques were needed to further investigate this issue. We have added these points and more thoroughly discussed the potential mechanism in the revised text (see lines 329-339, 405-415, 418-420).

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> *(2) I also found no systematic discussion and theoretical contributions regarding the correlation with the autistic traits. If the main point of this paper is to highlight an*

> *implicit and objective behavioral marker of the autistic trait, more interpretation and discussion of the links between the results and existing findings in ASD are needed.*

We thank the reviewer for this insightful suggestion. The perception of biological motion (BM) has long been considered an important hallmark of social cognition. Abundant studies reported that individuals with social cognitive deficits (e.g., ASD) were impaired in BM perception (Blake et al., 2003; Freitag et al., 2008; Klin et al., 2009; Nackaerts et al., 2012). More recently, it has been pointed out that the extraction of more complex social information (e.g., emotions, intentions) from BM, as compared to basic BM recognitions, could be more effective in detecting ASDs (Federici et al., 2020; Koldewyn et al., 2009; Parron et al., 2008; Todorova et al., 2019). Specifically, a meta-analysis found that the effect size expanded nearly twice when the task required emotion recognition as compared to simple perception/detection (Todorova et al., 2019). However, for the high-functioning ASD individuals, it has been reported that they showed comparable performance with the control group in explicitly labelling BM emotions, while their responses were rather delayed (Mazzoni et al., 2021). This suggested that ASD individuals could adopt compensatory strategies to complete the explicit BM labelling task, while their automatic behavioural responses remained impaired. This highlights the importance of using more objective measures that do not rely on active reports to investigate the intrinsic perception of emotions from BM and its relationship with ASD-related social deficits. The current study thus introduced the pupil size measurement to this field, and we combined it with the passive viewing task to investigate the more automatic aspect of BM emotion processing. More importantly, in addition to diagnostic ASDs, the non-clinical general population also manifested autistic tendencies that followed normal distribution and demonstrated substantial heritability (Hoekstra et al., 2007). Here, we focused on the autistic tendencies in the general population, and our results showed that pupil modulations by BM emotions were indicative of individual autistic traits. Specifically, passively viewing the happy BMs evoked larger pupil responses than the sad BMs, while such emotional modulation diminished with the increase of autistic tendencies. More detailed test-retest examination further illustrated such a correlation was driven by the general diminishment in pupil modulation effects by emotional BM (happy or sad) for individuals with high autistic tendencies. This finding demonstrated that the automatic emotion processing of BM stimuli was impaired in individuals with high autistic tendencies, lending support to previous studies (Hubert et al., 2006; Nackaerts et al., 2012; Parron et al., 2008). This indicated the utility of emotional BM stimuli and pupil measurement in identifying ASD-related tendencies in both clinical and non-clinical populations. We have added these points to the revised text (see lines 347-375).

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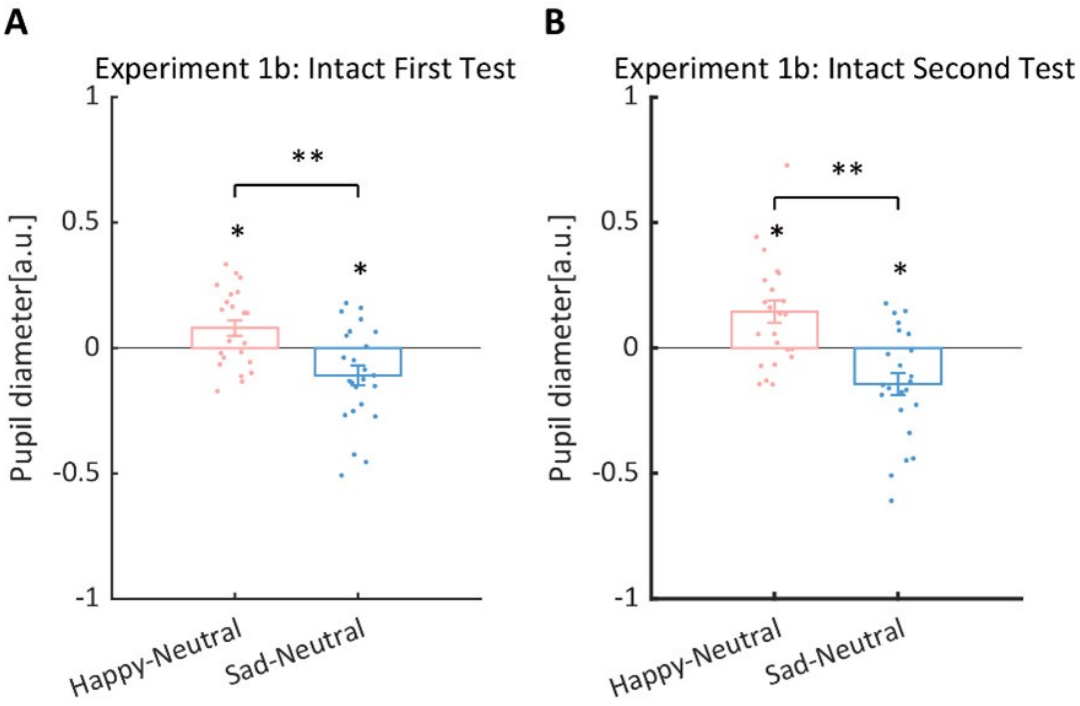
> ***Reviewer #2 (Public Review):***
>
> *Summary:*
>
> *Through a series of four experiments, Yuan, Wang and Jiang examined pupil size responses to emotion signals in point-light motion stimuli. Experiment 1 examined upright happy, sad and neutral point-light biological motion (BM) walkers. The happy BM induced a significantly larger pupil response than the neutral, whereas the sad BM evoked a significantly smaller pupil size than the neutral BM. Experiment 2 examined inverted BM walkers. Experiment 3 examined BM stimuli with acceleration removed. No significant effects of emotion were found in neither Experiment 2 nor Experiment 3. Experiment 4 examined scrambled BM stimuli, in which local motion features were preserved while the global configuration was disrupted. Interestingly, the scrambled happy and sad BM led to significantly greater pupil size than the scrambled neutral BM at a relatively early time, while no significant difference between the scrambled happy and sad BM was found. Thus, the authors argue that these results suggest multi-level processing of emotions in life motion signals.*
>
> *Strengths:*
>
> *The experiments were carefully designed and well-executed, with point-light stimuli that eliminate many potential confounding effects of low-level visual features such as luminance, contrast, and spatial frequency.*
>
> *Weaknesses:*
>
> *Correlation results with limited sample size should be interpreted with extra caution.*

Thanks for pointing this out. To strengthen the correlation results, we have conducted a replication experiment (Exp.1b) and added a test-retest examination to further assess the reliability of our measurements. Specifically, a new group of 24 participants (16 females, 8 males) were recruited to perform the identical experiment procedure as in Experiment 1. Then, after at least seven days, they were asked to return to the lab for a retest. The results successfully replicated the previously reported main effect of emotional condition in both the first test ($F(2, 46) = 12.0$, $p < .001$, $\eta p2 = 0.34$, Author response image 1A) and the second test ($F(2, 46) = 14.8$, $p < .001$, $\eta p2 = 0.39$, Author response image 1B). The happy BM induced a significantly larger pupil response than the neutral BM (First Test: $t(23) = 2.60$, $p = .022$, Cohen's $d = 0.53$, 95% CI for the mean difference = [0.02, 0.14], Holm-corrected, $p = .048$ after Bonferroni correction, Author response image 1A; Second Test: $t(23) = 3.36$, $p = .005$, Cohen's $d = 0.68$, 95% CI for the mean difference = [0.06, 0.24], Holm-corrected, $p = .008$ after Bonferroni correction, Author response image 1B). On the contrary, the sad BM induced a significantly smaller pupil response than the neutral BM (First Test: $t(23) = -2.77$, $p = .022$, Cohen's $d = 0.57$, 95% CI for the mean difference = [-0.19, -0.03], Holm-corrected, $p = .033$ after Bonferroni correction; Second Test: $t(23) = -3.19$, $p = .005$, Cohen's $d = 0.65$, 95% CI for the mean difference = [-0.24, -0.05], Holm-corrected, $p = .012$ after Bonferroni correction, Author response image 1B). Besides, the happy BM induced significantly larger pupil response than the sad BM (first test: $t(23) = 4.23$, $p < .001$, Cohen's $d = 0.86$, 95% CI for the mean difference = [0.10, 0.28], Holm-corrected, $p < .001$ after Bonferroni correction, Author response image 1A; second test: $t(23) = 4.26$, $p < .001$, Cohen's $d = 0.87$, 95% CI for the mean difference = [0.15, 0.44], Holm-corrected, $p < .001$ after Bonferroni correction, Author response image 1B). The results of the cluster-based permutation analysis were also similar (see Supplementary Material for more details).

Author response image 1.

Normalized mean pupil responses in the replication experiment (Experiment 1b) of Experiment 1a and its retest, using the neutral condition as baseline, plotted against happy and sad conditions. (A) In the first test, the group average pupil response to happy intact BM is significantly larger than that to sad and neutral BM, while the pupil response induced by sad BM is significantly smaller than that evoked by neutral BM, replicating the results of Experiment 1a. (B) Moreover, such results were similarly found in the second test.



Notably, we successfully replicated the negative correlation between the happy over sad dilation effect and individual autistic traits in the first test (r(23) = -0.46, p = .023, 95% CI for the mean difference = [-0.73, -0.07], Author response image 2A). No other significant correlations were found (see Author response image 2B-C). Moreover, in the second test, such a correlation was similarly found and was even stronger (r(23) = -0.61, p = .002, 95% CI for the mean difference = [-0.81, -0.27], Author response image 2D). We've also performed a test-retest reliability analysis on the happy over sad pupil dilation effect and the AQ score. The results showed robust correlations. See Author response table 1 for more details.

Author response table 1.

Reliability of pupil size and AQ indices.

Measurements	First Test	Second Test	Test-retest	
	M(SD)		*α*	*r*
happy-sad pupil size	0.19(0.22)	0.30(0.34)	0.60	0.61 **
AQ score	19.4(5.6)	19.9(6.2)	0.82	0.90 ***

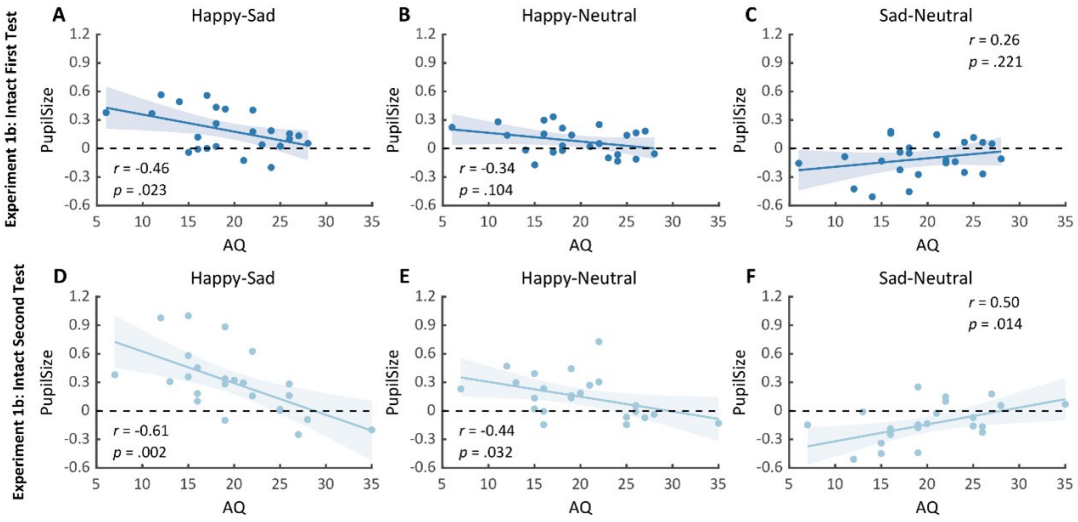
Note. α = Cronbach's α; *r* = Pearson's r, *** $p < .001$, ** $p < .01$

Importantly, in the second test, we've also observed a significant negative correlation between AQ and the happy minus neutral pupil dilation effect (r(23) = -0.44, p = .032, 95% CI for the mean difference = [-0.72, -0.04], Author response image 2E), and a significant positive correlation between the sad minus neutral pupil size and AQ (r(23) = 0.50, p = .014, 95% CI for the mean difference = [0.12, 0.75], Author response image 2F). This indicated that the overall correlation between happy over sad dilation effect and AQ was driven both by the diminished happy dilation effect as well as the sad constriction effect. Overall, our replication experiment consistently found a significant negative correlation between AQ and happy over sad dilation effect both in the test and the retest. Moreover, it revealed that such an effect was contributed by both a negative correlation between AQ and happy-neutral pupil response and a positive correlation between AQ and sad-neutral pupil response, demonstrating a general impairment in BM emotion perception (happy or sad) for individuals with high autistic tendencies. This also indicated the utility of adopting a test-retest pupil examination

to more precisely detect individual autistic tendencies. We have added these points in the revised text (see lines 135-173, lines 178-180).

Author response image 2.

Correlation results for pupil modulation effects and AQ scores in the replication experiment (Experiment 1b) of Experiment 1a and its retest. (A) We replicated the negative correlation between the happy over sad pupil dilation effect and AQ in the first test. (B-C) No other significant correlations were found. (D) In the second test, the negative correlation between the happy over sad pupil dilation effect and AQ was similarly observed and even stronger. (E-F) Moreover, the happy vs. neutral pupil dilation effect and the sad vs. neutral pupil constriction effect respectively correlate with AQ in the second test.



> *It would be helpful to add discussions as a context to compare the current results with pupil size reactions to emotion signals in picture stimuli.*

Thanks for this this thoughtful comment. The modulation of emotional information on pupil responses has been mostly investigated using picture stimuli. Bradley et al. (2008) first demonstrated that humans showed larger pupil responses towards emotional images as compared to neutral images, while no difference was observed between the positive and negative images. This was regarded as the result of increased sympathetic activity induced by emotional arousal that is independent of the emotional valence. Similar results have been replicated with different presentation durations, repetition settings, and tasks (Bradley & Lang, 2015; Snowden et al., 2016). However, the emotional stimuli adopted in these studies were mostly complicated scene images that conveyed rather general emotional information. When it comes to the specific emotion cues (e.g., fear, anger, happy, sad) delivered by our conspecifics through biologically salient signals (e.g., faces, gestures, voices), the results became intermixed. Some studies demonstrated that fearful, disgusted, and angry static faces induced larger pupil sizes than the neutral face, while sad and happy faces failed to induce such pupil dilatory effects (Burley et al., 2017). In contrast, other studies observed larger pupil responses for happy faces as compared to sad and fearful faces (Aktar et al., 2018; Burley & Daughters, 2020; Jessen et al., 2016). These conflicting results could be due to the low-level confounds of emotional faces (e.g., eye size) (Carsten et al., 2019; Harrison et al., 2006). Similar to faces, BM also conveyed salient clues concerning the emotional states of our interactive partners. However, they were highly simplified, deprived of various irrelevant visual confounders (e.g., body shape). Here, we reported that the happy BM induced a stronger pupil response than the neutral and sad BM, lending support to the happy dilation

effect observed with faces (Burley & Daughters, 2020; Prunty et al., 2021). Moreover, it helps ameliorate the concern regarding the low-level confounding factors by identifying similar pupil modulations in another type of social signal with distinctive perceptual features. We have added these points to the revised text (see lines 301-321).

References:

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> *Overall, I think this is a well-written paper with solid experimental results that support the claim of the authors, i.e., the human visual system may process emotional information in biological motion at multiple levels. Given the key role of emotion processing in normal social cognition, the results will be of interest not only to basic scientists who study visual perception, but also to clinical researchers who work with patients of social cognitive disorders. In addition, this paper suggests that examining pupil size responses could be a very useful methodological tool to study brain mechanisms underlying emotion processing.*

Reviewer #3 (Public Review):

Summary:

The overarching goal of the authors was to understand whether emotional information conveyed through point-light biological motion can trigger automatic physiological responses, as reflected in pupil size.

Strengths:

This manuscript has several noticeable strengths: it addresses an intriguing research question that fills that gap in existing literature, presents a clear and accurate presentation of the current literature, and conducts a series of experiments and control experiments with adequate sample size. Yet, it also entails several noticeable limitations - especially in the study design and statistical analyses.

Weaknesses:

(1) Study design:

(1.1) Dependent variable:

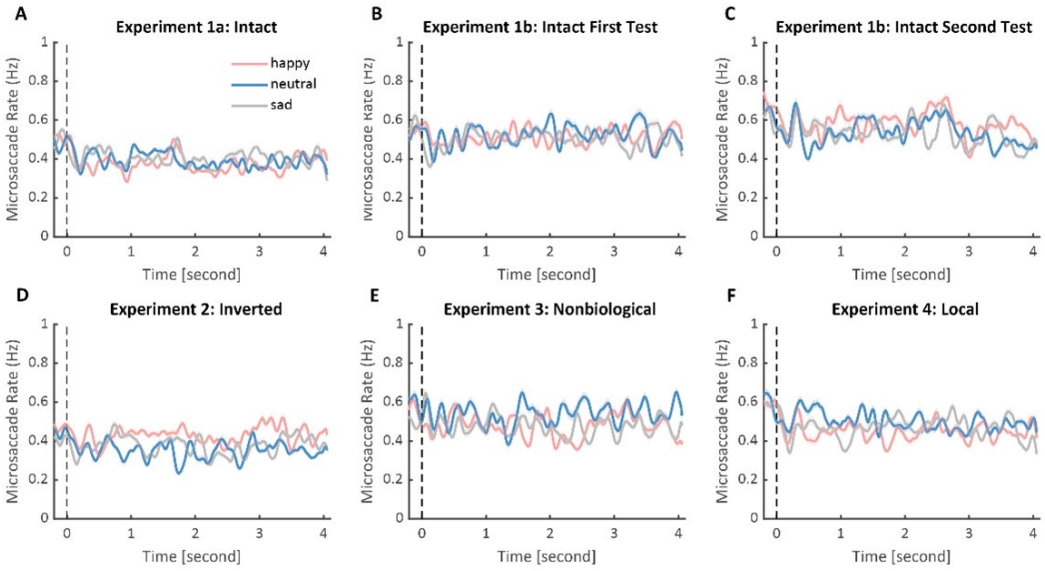
Emotional attention is known to modulate both microsaccades and pupil size. Given the existing pupillometry data that the authors have collected, it would be both possible and valuable to determine whether the rate of microsaccades is also influenced by emotional biological motion.

We thank the reviewer for this advice. Microsaccades functioned as a mechanism to maintain visibility by continuously shifting the retinal image to overcome visual adaptation (Martinez-Conde et al., 2006). Moreover, it was found to be sensitive to attention processes (Baumeler et al., 2020; Engbert & Kliegl, 2003b; Meyberg et al., 2017), and could reflect the activity of superior colliculus (SC) and other related brain areas (Martinez-Conde et al., 2009, 2013). Previous studies have found that, compared with neutral and pleasant images, unpleasant images significantly inhibit early microsaccade rates (Kashihara, 2020; Kashihara et al., 2013). This is regarded as the result of retaining previous crucial information at the sacrifice of updating new visual input. We agree with the reviewer that it would be valuable to investigate whether emotional information conveyed by BM could modulate microsaccades. However, it should be noted that our data collection and experimental design are not optimized for this purpose. This is because we have only recorded the left eye's data, while abundant methodological studies have doubted the reliability of using only one eye's data to analyze microsaccades (Fang et al., 2018; Hauperich et al., 2020; Nyström et al., 2017) and suggested that the microsaccades should be defined by spontaneous binocular eye movement (Engbert & Kliegl, 2003a, 2003b). Besides, according to Kashihara et al. (2013), participants showed differential microsaccade rates after the stimuli disappeared so as to maintain the previously observed different emotional information. However, in the current study, we discarded the data after the stimuli disappeared, making it impossible to analyze the microsaccade data after the stimuli disappeared. Despite these disadvantages, we have attempted to analyze the microsaccade rate during the stimuli presentation using only the left eye's data. Specifically, we applied the algorithm developed by Otero-Millan et al. (2014) (minimum duration =6 ms, maximum amplitude = 1.5 degrees, maximum velocity = 150 degrees/sec) to the left eye's data from 100 ms before to 4000 ms after stimulus onset. Subsequently, we calculated the microsaccade rates using a moving window of 100 ms (stepped in 1 ms) (Engbert & Kliegl, 2003b; Kashihara et al., 2013). The microsaccade rate displayed a typical curve, with suppression shortly after stimulus appearance (inhibition phase), followed by an increased rate of microsaccade occurrence (rebound phase). The cluster-based permutation analysis was then applied to explore the modulation of BM

emotions on microsaccade rates. However, no significant differences among different emotional conditions (happy, sad, neutral) were found for the four experiments.

Author response image 3.

Time-series change in the microsaccade rates to happy, sad, and neutral BM in Experiments 1-4. Solid lines represent microsaccade rates under each emotional condition as a function of time (happy: red; sad: blue; neutral: gray); shaded areas represent the SEM between participants. No significant differences were found after cluster-based permutation correction for the four experiments.



It is important to note that the microsaccade rate analysis was conducted on only the left eye's data and that the experiment design is not optimized for this analysis, thus, extra caution should be exercised in interpreting the results. Still, we found it very innovative and important to combine the microsaccade index with the pupil size to holistically investigate the processing of emotional information in BM, and future studies are highly needed to adopt more suitable recording techniques and experiment designs to further probe this issue. We have discussed this issue in the revised text (see lines 339-344).

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> *(1.2) Stimuli:*
>
> *It appears that the speed of the emotional biological motion stimuli mimics the natural pace of the emotional walker. What is the average velocity of the biological motion stimuli for each condition?*

Thanks for pointing out this issue. The neutral and emotional (sad or happy) BM stimuli are equal in walking speed (one step for one second, 1Hz). We have also computed their physical velocity by calculating the Euclidean distance in pixel space of each key point between adjacent frames (Poyo Solanas et al., 2020). The velocity was 5.76 pixels/frame for the happy BM, 4.14 pixels/frame for the neutral BM, and 3.21 pixels/frame for the sad BM. This difference in velocity profile was considered an important signature for conveying emotional information, as the happy walker was characterized by a larger step pace and longer arm swing and the sad walker would instead exhibit a slouching gait with short slow strides and smaller arm movement (Barliya et al., 2012; Chouchourelou et al., 2006; Halovic & Kroos, 2018; Roether et al., 2009). More importantly, our current results could not be explained by the differences in velocities. This is because the inverted emotional BM with identical velocity characteristics failed to induce any modulations on pupil responses. Furthermore, the local sad and happy BM differed the most in velocity feature, while they induced similar modulations on pupil sizes. We have added these points in the revised text (see lines 254-257, 484-491).

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> *When the authors used inverted biological motion stimuli, they didn't observe any modulation in pupil size. Could there be a difference in microsaccades when comparing inverted emotional biological motion stimuli?*

Thanks for this consideration. Both microsaccades and pupil size can provide valuable insights into the underlying neural dynamics of attention and cognitive control (Baumeler et al., 2020; Engbert & Kliegl, 2003; Meyberg et al., 2017). Notably, previous studies have shown that the microsaccades and pupil sizes could be similar and highly correlated in reflecting various cognitive processes, such as multisensory integration, inhibitory control, and cognitive load (Krejtz et al., 2018; Wang et al., 2017; Wang & Munoz, 2021). Moreover, the generation of both microsaccades and pupil responses would involve shared neural circuits, including the midbrain structure superior colliculus (SC) and the noradrenergic system (Hafed et al., 2009; Hafed & Krauzlis, 2012; Wang et al., 2012). However, the pupil size could be more sensitive than microsaccade rates in contexts such as affective priming (Krejtz et al., 2020) and decision formation (Strauch et al., 2018). Moreover, abundant former studies have all shown that inversion would significantly disrupt the perception of emotions from BM (Atkinson et al., 2007; Dittrich et al., 1996; Spencer et al., 2016; Yuan et al., 2022, 2023). Overall, it is unlikely for the microsaccade rates to show significant differences when comparing inverted emotional biological motion stimuli. Besides, we have attempted to analyze the microsaccade rate in the inverted BM situation, while our results showed no significant differences (see also Point 1.1, Author response image 3). Still, it is needed for future studies to combine the microsaccade index and pupil size to provide a thorough understanding of BM emotion processing. We have discussed this issue in the revised text (see lines 339-344).

References:

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> *(2) Statistical analyses*
>
> *(2.1) Multiple comparisons:*
>
> *There are many posthoc comparisons throughout the manuscript. The authors should consider correction for multiple comparisons. Take Experiment 1 for example, it is important to note that the happy over neutral BM effect and the sad over neutral BM effect are no longer significant after Bonferroni correction, which is worth noting.*

Thanks for this suggestion. In our original analysis, we applied the Holm post-hoc corrections for multiple comparisons. The Holm correction is a step-down correction method and is more

powerful but less conservative than the Bonferroni correction. We have now conducted the stricter Bonferroni post-hoc correction. In Experiment 1, the happy over neutral, and happy over sad BM effect is still significant after the Bonferroni post-hoc correction (happy vs. neutral: p = .036; happy vs. sad: p = .009), and the sad over neutral comparison remains marginally significant after the Bonferroni post-hoc correction (p = .071). Importantly, the test-retest replication experiment also yielded significant results for the comparisons between happy and neutral (First Test: p = .022, Holm-corrected, p = .048, Bonferroni-corrected; Second Test: p = .005, Holm-corrected, p = .008, Bonferroni-corrected), sad and neutral (First Test: p = .022, Holm-corrected, p = .033, Bonferroni-corrected; Second Test: p = .005, Holm-corrected, p = .012, Bonferroni-corrected, Author response image 1B), and happy and sad BM (First test: p < .001, Holm-corrected, p < .001, Bonferroni-corrected; Second test: p < .001, Holm-corrected, p < .001, Bonferroni-corrected). These results provided support for the replicability and consistency of the reported significant contrasts. See also Point 2.3.

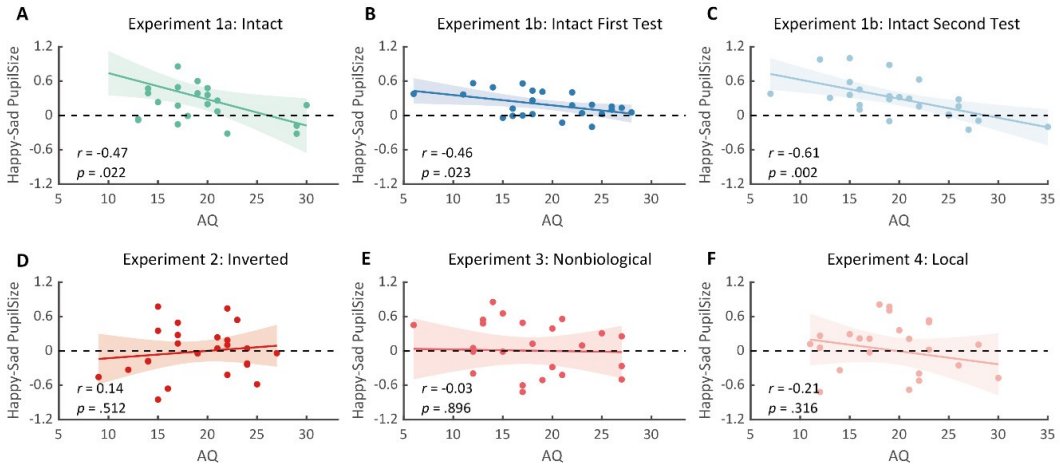
In Experiment 4, the significance levels of all comparisons remained the same after Bonferroni post-hoc correction (happy vs. neutral: p = .011; sad vs. neutral: p = .007; happy vs. sad: p = 1.000). We have now added these results in the main text (See lines 119, 122, 124, 143, 145, 148, 150, 153, 155, 248, 251, 254).

> *(2.2) The authors present the correlation between happy over sad dilation effect and the autistic traits in Experiment 1, but do not report such correlations in Experiments 2-4. Did the authors collect the Autistic Quotient measure in Experiments 2-4? It would be informative if the authors could demonstrate the reproducibility (or lack thereof) of this happy-sad index in Experiments 2-4.*

We apologize for not making it clear. We have collected the AQ scores in Experiments 2-4. However, it should be pointed out that the happy over sad pupil dilation effect was only observed in Experiment 1. Moreover, we've again identified such happy over sad pupil dilation effect in the replication experiment (Experiment 1b) as well as its correlation with AQ. Instead, no significant correlations between AQ and the happy-sad pupil index were found in Experiments 2-4, see Author response image 4 for more details. We have reported these correlations in the main text (see lines 157-173, 190-194, 212-216, 257-262).

Author response image 4.

Correlations between the happy over sad pupil dilation effect and AQ scores. (A) The happy over sad pupil dilation effect correlated negatively with individual autistic scores. (B-C) Such correlation was similarly observed in the test and retest of the replication experiment. (D-F) No such correlations were found for the inverted, nonbiological, and local BM stimuli.



> *(2.3) The observed correlation between happy over sad dilation effect and the autistic traits in Experiment 1 seems rather weak. It could be attributed to the poor reliability of the Autistic Quotient measure or the author-constructed happy-sad index. Did the authors examine the test-retest reliability of their tasks or the Autistic Quotient measure?*

Thanks for this suggestion. We have now conducted a test-retest replication study to further confirm the observed significant correlations. Specifically, we recruited a new group of 24 participants (16 females, 8 males) to perform the identical procedure as in Experiment 1, and they were asked to return to the lab for a retest after at least seven days. We've replicated the significant main effect of emotional conditions in both the first test ($F(2, 46) = 12.0$, $p < .001$, $\eta p2 = 0.34$) and the second test ($F(2, 46) = 14.8$, $p < .001$, $\eta p2 = 0.39$). Besides, we also replicated the happy minus neutral pupil dilation effect (First Test: $t(23) = 2.60$, $p = .022$, Cohen's $d = 0.53$, 95% CI for the mean difference = [0.02, 0.14], Holm-corrected, $p = .048$ after Bonferroni correction; Second Test: $t(23) = 3.36$, $p = .005$, Cohen's $d = 0.68$, 95% CI for the mean difference = [0.06, 0.24], Holm-corrected, $p = .008$ after Bonferroni correction), and the sad minus neutral pupil constriction effect (First Test: $t(23) = -2.77$, $p = .022$, Cohen's $d = 0.57$, 95% CI for the mean difference = [-0.19, -0.03], Holm-corrected, $p = .033$ after Bonferroni correction; Second Test: $t(23) = -3.19$, $p = .005$, Cohen's $d = 0.65$, 95% CI for the mean difference = [-0.24, -0.05], Holm-corrected, $p = .012$ after Bonferroni correction). Additionally, the happy BM still induced a significantly larger pupil response than the sad BM (first test: $t(23) = 4.23$, $p < .001$, Cohen's $d = 0.86$, 95% CI for the mean difference = [0.10, 0.28], Holm-corrected, $p < .001$ after Bonferroni correction; second test: $t(23) = 4.26$, $p < .001$, Cohen's $d = 0.87$, 95% CI for the mean difference = [0.15, 0.44], Holm-corrected, $p < .001$ after Bonferroni correction).

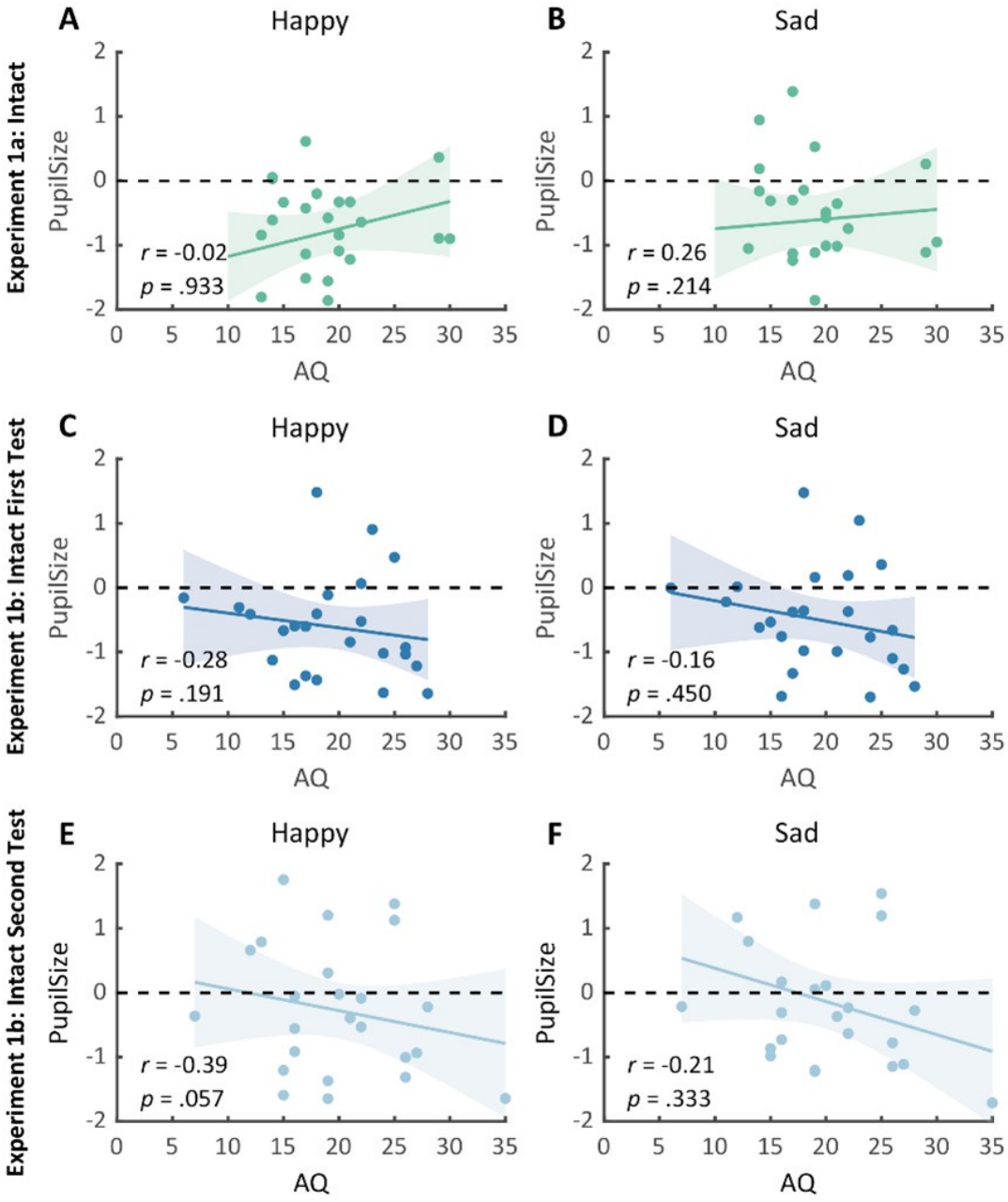
Notably, we've successfully replicated the negative correlation between the happy over sad dilation effect and individual autistic traits ($r(23) = -0.46$, $p = .023$, 95% CI for the mean difference = [-0.73, -0.07]). Such a correlation was similarly found and was even stronger in the retest ($r(23) = -0.61$, $p = .002$, 95% CI for the mean difference = [-0.81, -0.27]). A test-retest reliability analysis was conducted on the happy over sad pupil dilation effect and the AQ score. The results showed robust correlations (r(happy-sad pupil size)= 0.56; r(AQ)= 0.90) and strong test-retest reliabilities (α(happy-sad pupil size)= 0.60; α(AQ)= 0.82). We have added these results to the main text (see lines 135-173). See also Response to Reviewer #2 Response 1 for more details.

> *(2.4) Relatedly, the happy over sad dilation effect is essentially a subtraction index. Without separately presenting the pipul size correlation with happy and sad BM in supplemental figures, it becomes challenging to understand what's primarily driving the observed correlation.*

Thanks for pointing this out. We have now presented the separate correlations between AQ and the pupil response towards happy and sad BM in Experiment 1 (see Author response image 5A), and the test-retest replication experiment of Experiment 1 (see Author response image 5B-C). No significant correlations were found. This is potentially because the raw pupil response is a mixed result of BM perception and emotion perception, while the variations in pupil sizes across emotional conditions could more faithfully reflect individual sensitivities to emotions in BM (Burley et al., 2017; Pomè et al., 2020; Turi et al., 2018).

Author response image 5.

No significant correlations between AQ and pupil response towards happy and sad intact BM were found in Experiment 1a and the test-retest replication experiment (Experiment 1b).

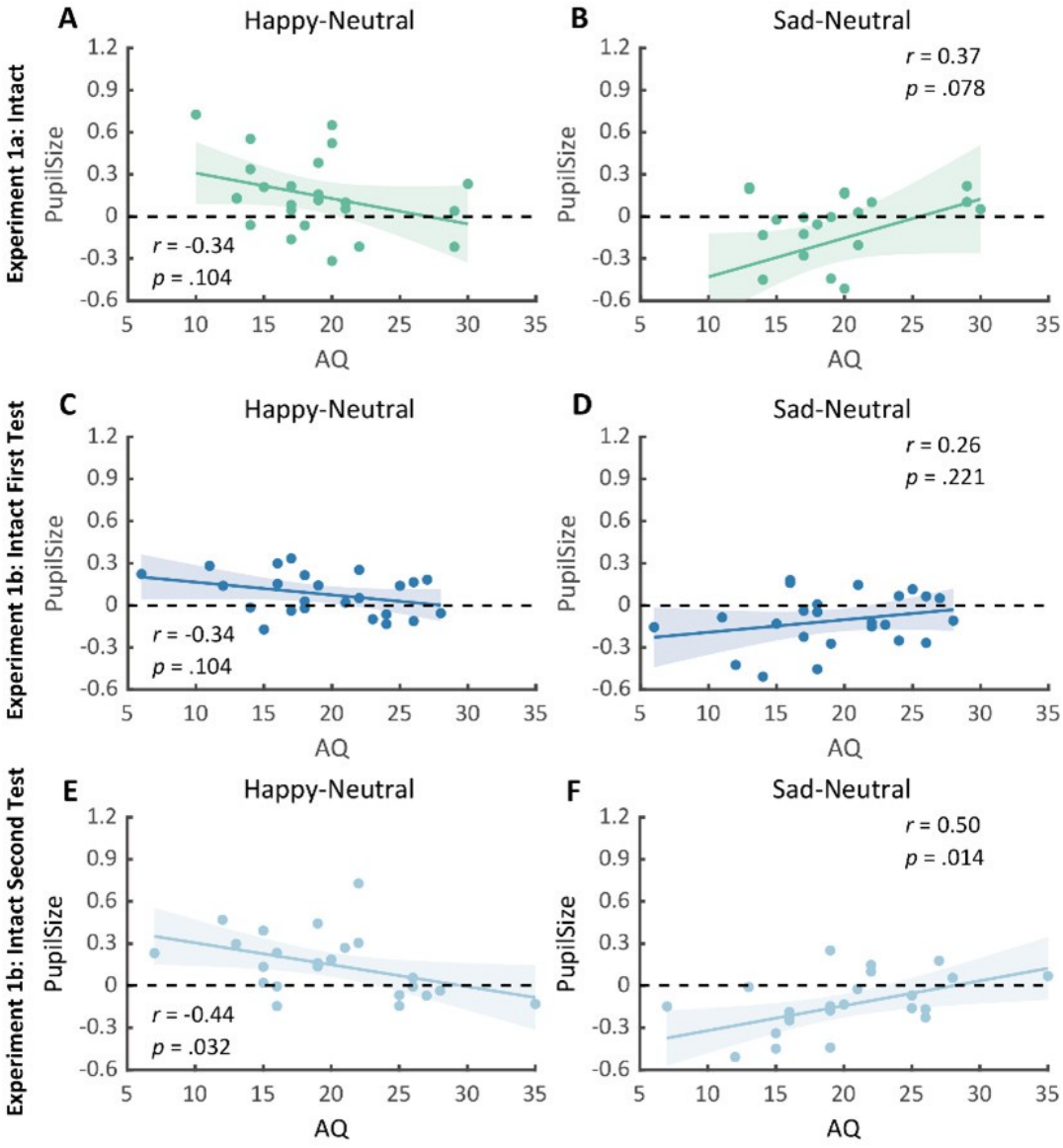


To probe what's primarily driving the observed correlation between happy-sad pupil size and AQ, we instead used the neutral as the baseline and separately correlated AQ with the happy-neutral and the sad-neutral pupil modulation effects. No significant correlation was found in Experiment 1a (Author response image 6A-B) and the first test of the replication experiment (Experiment 1b) (Author response image 6C-D). Importantly, in the second test of the replication experiment, we found a significant negative correlation between AQ and the happy-neutral pupil size ($r(23) = -0.44$, $p = .032$, 95% CI for the mean difference = [-0.72, -0.04], Author response image 6E), and a significant positive correlation between AQ and the sad-neutral pupil size ($r(23) = 0.50$, $p = .014$, 95% CI for the mean difference = [0.12, 0.75], Author response image 6F). This suggested that the overall correlation between AQ and the happy over sad dilation effect was driven by diminished pupil modulations towards both the happy and sad BM for high AQ individuals, demonstrating a general deficiency in BM emotion perception (happy or sad) among individuals with high autistic tendencies. It further

revealed the potential of adopting a test-retest pupil examination to more precisely detect individual autistic tendencies. We have reported these results in the main text (see lines 166-173).

Author response image 6.

Correlation results for pupil modulations and AQ scores. (A-B) In Experiment 1a, no significant correlation was observed between AQ and the happy pupil modulation effect, as well as between AQ and the sad pupil modulation effect. (C-D) Similarly, no significant correlations were found in the first test of the replication experiment (Experiment 1b). (E-F) Importantly, in the second test of Experiment 1b, the happy vs. neutral pupil dilation effect was positively correlated with AQ, and the sad vs. neutral pupil constriction effect was positively correlated with AQ.



References:

Burley, D. T., Gray, N. S., & Snowden, R. J. (2017). As Far as the Eye Can See: Relationship between Psychopathic Traits and Pupil Response to Affective Stimuli. PLOS ONE, 12(1), e0167436. https://doi.org/10.1371/journal.pone.0167436

Pomè, A., Binda, P., Cicchini, G. M., & Burr, D. C. (2020). Pupillometry correlates of visual priming, and their dependency on autistic traits. Journal of vision, 20(3), 3-3.

Turi, M., Burr, D. C., & Binda, P. (2018). Pupillometry reveals perceptual differences that are tightly linked to autistic traits in typical adults. eLife, 7. https://doi.org/10.7554/elife.32399

> *(2.5) For the sake of transparency, it is important to report all findings, not just the positive results, throughout the paper.*

Thanks for this suggestion. We have now reported all the correlations results between AQ and pupil modulation effects (happy-sad, happy-neutral, sad-neutral) in the main text (see lines 130-131, 157-162, 166-170, 190-194, 212-216, 257-262). Given that no significant correlations were observed between AQ and the raw pupil responses across four experiments, we reported their correlations with AQ in the supplementary material. We have stated this point in the main text (see lines 132-134).

> *(3) Structure*
>
> *(3.1) The Results section immediately proceeds to the one-way repeated measures ANOVA. This section could be more reader-friendly by including a brief overview of the task procedures and variables, e.g., shifting Fig. 3 to this section.*

Thanks for this advice. We have now added a brief overview of the task procedures and variables and we have also shifted the figure position (see lines 101-103).

> ***Reviewer #1 (Recommendations For The Authors):***
>
> *(1) I suggest that the authors first explain the task (i.e., Fig. 3) at the beginning of the results. And it seems more appropriate to show the time course figures (Fig. 2) and before the bar plots (Fig. 1). If I understand correctly, the bar plots reflect the averaged data from the time course plots. Also, please clearly state the time window used to average the data. The results of the correlation analysis can be displayed in the last step.*

Thanks for this suggestion. We have now added a concise explanation of the task at the beginning of the results (see lines 101-103). We have also adjusted the figure positions and adjusted the order of our results according to the reviewer's suggestion. The time window we used to average the data was from the onset of the stimuli until the end of the stimuli presentation. We have now clearly stated these issues in the revised text (see lines 111-112).

> *(2) According to the above, I think a more reasonable arrangement should be Fig. 3, 2, and 1.*

Thanks for this suggestion. We have adjusted the figure positions accordingly.

> *(3) Please include each subject's data points in the bar plots in Fig. 1.*

We have now presented each subject's individual data point in the bar plot.

> *(4) Lines 158-160 and 199-202 report interaction effects of the two-way ANOVA. This is good, but the direction of interaction effect should also be reported.*

We thank the reviewer for this suggestion. We have now reported the direction of the interaction effect. The significant interaction observed across Experiment 1 and Experiment 2 was mainly due to the diminishment of emotional modulation in inverted BM. The significant

interaction crossing Experiment 1 and Experiment 3 was similarly caused by the lack of emotional modulation in nonbiological stimuli. With regard to the significant interaction across Experiment 1 and Experiment 4, it could be primarily attributed to the vanishment of pupil modulation effect between happy and sad local BM. We have specified these points in the revised text, see lines 198-199, 219-220, 267-269.

> ***Reviewer #3 (Recommendations For The Authors):***
>
> *(1) Number of experiments:*
>
> *As stated in the Methods section, this study seems to consist of five experiments (120/24=5) according to the description below. However, the current manuscript only reports findings from four of these experiments. Can the authors clarify on this matter?*
>
> *"A total of 120 participants (44 males, 76 females) ranging from 18 to 29 years old (M ± SD = 23.1 ± 2.5) were recruited, with 24 in each experiment."*

We apologize for not making it clear. This referred to a pure behavior explicit emotion classification experiment (N=24) that served as a prior test to confirm that the local BM stimuli conveyed recognizable emotional information. We have now more carefully stated this issue in the revised text, see lines 456-458.

> *(2) Emotion processing mechanism of BM*
>
> *"Mechanism" is a very strong word, suggesting a causal relationship. In the setting of a passive viewing task that lacks any behavioral report, it is possible that the observed changes in pupil size could be epiphenomenal, rather than serving as the underlying mechanism.*

Thanks for this suggestion. We have now either changed “mechanism” into “phenomenon” or deleted it. We have also carefully discussed the potential implications for future studies to incorporate variant behavioral, physiological and neural indexes to yield more robust causal evidence to unveil the potential mechanism serving the observed multi-level BM emotion processing phenomenon.

> *(3) Data sharing*
>
> *The authors could improve their efforts in promoting data transparency to ensure a comprehensive view of the results. This implies sharing deidentified raw data instead of summary data in an Excel spreadsheet.*

Thanks for this suggestion. We have now uploaded the deidentified raw data. (https://doi.org/10.57760/sciencedb.psych.00125).

https://doi.org/10.7554/eLife.89873.2.sa0