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A longitudinal analysis of the development of infant facial expressions in response to acute pain: Immediate and regulatory expressions

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ABSTRACT

Facial expressions during infancy are important to examine, as infants do not have the language skills to describe their experiences. This is particularly vital in the context of pain, where infants depend solely on their caregivers for relief. The objective of the current study was to investigate the development of negative infant facial expressions in response to immunization pain over the first year of life. Infant facial expressions were examined longitudinally using a subsample of 100 infants that were each videotaped during their 2-, 4-, 6-, and 12-month routine immunization appointments. Infant facial expressions were coded using BabyFACS (facial action coding system) for the first minute after a painful needle prick. Facial expressions were examined with a catalogue of the most commonly occurring facial expressions. Results demonstrated that clear differences were seen over ages. Infants display a variety of facial expressions with some of the components of adult pain expressions immediately after the needle and they abate shortly after. However, infants did not display adult expressions of discrete negative emotions. Instead, infants displayed a variety of generalized pain and distress faces aimed at gaining caregiver aid. The development of nonverbal communication in infants, particularly facial expressions, remains an important area of inquiry. Further study into accurately measuring infant negative emotions, pain, and distress is warranted.

1. Introduction

The development of negative emotions in a pain context is an important area of study because the underpinnings of regulation from distress (pain-related or otherwise) occur in the first year of life [4]. Researchers have been divided between two major perspectives. According to Differential Emotions Theory, infants show clearly differentiated facial expressions of discrete negative emotions such as anger, fear, and pain from early infancy [13,15]. On the other hand, according to classic differentiation theories, facial expressions of specific, discrete negative emotions gradually differentiate from more generalized, global distress expressions [5,7,33]. In this vein, Oster's more recent ontogenetic perspective holds that infant facial and vocal expressions are neither precocious, immature adult expressions nor global and diffuse signs of arousal. Instead, they are distinctive behavioral adaptations that evolved because they serve crucial communicative functions in infancy [21].

The current study contributes to our knowledge of the development of negatively valenced facial expressions and emotions related to pain-related distress by following a group of infants undergoing routine immunizations. Immunizations allow us to examine infant facial expressions in response to a distressing stimulus in an ecologically valid setting. However, previous work studying presumed discrete emotions in the immunization context is limited by the use of coding systems that assume adult emotional prototypes and small sample sizes [16,17].

While we expected that we would observe a distinct pain face (validly and reliably measured by the well-established Neonatal Facial Coding System [11,12]), we did not limit our scope to only a pain face. Thus, if there were nonpain facial expressions present (eg, anger, fear) or variations of pain faces present, we wanted to be able to capture these constellations and their development over the first year of life. Only the Facial Action Coding System for Infants and Young Children (BabyFACS [20]) codes the full range

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of anatomical facial actions possible. Utilizing a comprehensive coding system, one that codes not only facial actions present in pain expressions but rather, the whole range of emotional facial displays, allows the opportunity to better understand the infant's emotional experience within the acute pain context. To our knowledge, this is the only extant study that uses this exhaustive system to track the development of facial expressions over the immunization appointment (ie, for 1 minute post needle) and over age (ie, at 2, 4, 6, and 12 months of age).

We aim to address 3 questions: (1) What are the most commonly occurring facial expressions after an acutely painful stimulus during the first year of life? (2) Does the average time spent in these different expressions change over infant age? (3) How does the occurrence of the commonly occurring facial expression change as infants regulate distress over the first minute after painful stimulus? We hypothesized that, in line with a differentiation model of emotions, pain-elicited facial expressions become more differentiated and complex with age. We also hypothesized that, specifically in line with the ontogenetic perspective, infant-specific facial expressions, distinct from prototypical adult expressions, would be found.

2. Methods

2.1. Participants

One hundred infants were randomly selected from a large cohort of infants being observed during 4 separate routine immunization appointments over the first year of life [23]. At the time of data analysis, 598 infants had been recruited into the cohort, with 131 infants having some video footage at all 4 appointments. Infants weighed, on average, 7.43 lbs (SD = 0.92) at birth, and were most often the first or second born child in the family (52% first born and 42% second born). Half of the sample was male (50%). Mothers were most often the primary caregiver and had an average age of 34.76 years (SD = 4.82). Mothers were most often married (87%) and educated (74% minimum university educated). Infants were also culturally diverse, with 83% of mothers reporting a heritage culture other than North American. Infants at the 2-, 4-, and 6-month immunization appointments most often received a Pediacel (Sanofi Pasteur, Maidenhead, Berkshire, UK) vaccine first, followed by a Pentacel (Sanofi Pasteur) vaccine. At the 12-month appointment, infants received a Pentacel vaccine and either a measles, mumps, and rubella vaccine or a Priorix (GlaxoSmithKline, Brentford, Middlesex, UK) vaccine. Refer to Table 1 for a detailed summary of demographic information.

2.2. Procedure

Ethical approval was obtained through research ethics review boards at both the participating university and the associated pediatric hospital. Briefly, healthy infants were observed during routine immunization appointments at 2, 4, 6, and 12 months of age. During these appointments, infants' facial expressions were video-recorded before and after the immunization. Caregivers completed demographic and health questionnaires at each visit. A detailed description of the procedures for the larger study is previously described and available for review [23].

2.3. Facial coding measure

Infant facial expressions were coded for 1 minute after the last immunization needle using BabyFACS [20]. BabyFACS is an adaptation for infants and young children of the adult Facial Action Coding System (FACS) [9,10] that accounts for the differences in infant facial morphology. It is a comprehensive, anatomically based measure of all visually discernable facial movements in infants. BabyFACS does not provide formulas for facial expressions of emotions, but rather provides a method to describe constellations of facial actions displayed without a priori assumptions of what facial actions may be displayed based on adult facial expressions. BabyFACS includes 73 facial muscle actions (each identified by a number) coded for intensity from A (trace) to E (maximum). This coding creates full-face scores that describe precisely which facial muscles are activated and how strongly they are activated. BabyFACS also includes 3-by-3 matrices for coding intensities and variants of smiles and cry faces. BabyFACS coding allows for descriptions of infant facial expressions without prior assumptions of their meaning, and therefore provides an objective measure of infant facial expressions. For the current analysis, coding was done in 2 stages. First, 100% of the footage was double-coded by certified BabyFACS coders to ensure an agreed-upon time for the onset, apex, and offset of each facial expression. Second, using these agreed-upon onset, apex, and offset times, footage was independently coded by the 2 coders on BabyFACS. Twenty-five percent of the data was double-coded for inter-rater reliability and, based on the FACS and BabyFACS manuals method, the reliability coefficient was deemed acceptable at 0.84.

2.4. Coding and analysis procedure

Infant facial expressions were coded for 1 minute after the final immunization needle of the appointment. Categories of facial expressions were determined in a stepwise manner led by the lead author in collaboration with a coauthor and then independently confirmed with the author of BabyFACS. Categories were explored via frequency of facial expressions across the sample of infants, as well as via duration of the facial expressions. Initial steps in creating categories included an examination of facial actions that could perhaps be clustered together due to anatomic similarity. In the eyes, Action Unit (AU)6 results in the raising of the cheeks and skin below the eyes, and AU7 results in squinting and tightening the lower eyelids; both narrow the eye opening. These 2 facial actions (AU6 and AU7) are difficult to distinguish in infants within the pain context and were clustered together. In the midface, AU9, AU10, and AU11 all deepen the nasolabial furrow and raise the upper lip; these facial actions often co-occur and can be difficult to distinguish in distress expressions. These 3 facial actions (AU9, AU10, and AU11) were therefore clustered together. In the mouth, AU26 and AU27 are related to how widely a mouth is vertically opened. In the context of a cry face, these facial actions both reflect a dropping of the jaw, and were clustered together. A subsequent review of the adult literature on facial expressions of pain confirmed that these clusters of facial actions were appropriate given our context [30]. Next, based on the potential conceptual importance of certain facial actions and sub-constellations (namely AU43, eyes closed; AU6 or AU7, cheek raise or tightening of the eyelid; AU20, horizontal stretch; cry mouth matrix [19,29]), the categories were further refined. Categories were collapsed and adapted to create fewer groupings with consideration of particular facial actions and cry mouth combinations. Seven categories of facial expression constellations of negative affect were agreed upon based on frequency, inclusivity, mutual exclusiveness, and observable differences of categories.

To avoid a priori assumptions regarding the interpretation of facial expressions during our initial coding and synthesis, the most commonly occurring facial configurations were randomly assigned a colour name. To answer our first question, we first describe a catalogue of each of 7 discerned facial expressions (Red, Orange, Yellow, Green, Blue, Purple, and Black) along with the mean percentage of time over the minute that infants spent displaying that

Table 1
Demographic variables and characteristics of participants (n = 100).

	Frequency	Percentage
Infant sex (% male)	50	50.00%
Parent-reported heritage culture		
North American	17	17.00%
European	47	47.00%
Asian	11	11.00%
South/Latin American	01	01.00%
Middle Eastern/African	02	02.00%
Jewish	08	08.00%
Other	08	08.00%
Mixed Canadian	06	06.00%
Mother's highest education		
Graduate school/professional training	30	30.00%
University graduate (4 years)	44	44.00%
Partial university (at least 1 year)	04	04.00%
Trade school/community college	16	16.00%
High school graduate	06	06.00%
Marital status		
Married	87	87.00%
Divorced/separated	02	02.00%
Never married	01	01.00%
Other	01	01.00%
Common law	08	08.00%
Engaged	01	01.00%

facial expression. To answer our second question, statistical analyses compared the average percentage of the minute each facial expression was displayed across age (2, 4, 6, and 12 months) using a related-samples Friedman's 2-way analysis of variance by ranks for each facial expression. A Bonferroni correction was applied such that $P < 0.001$ was required for significance. Significant analyses of variance were followed by simple pairwise comparisons. Finally, to answer the third question, the 1-minute postneedle epoch was broken up into 5-second increments, and the average percentage of time within each 5-second sub-epoch was graphed to describe how the occurrence of facial displays changed over the first minute after the last needle.

For discussion and interpretation of our findings, each general category (facial expression) was also coded based on the Neonatal Facial Coding System (NFCS [11,12]), the Maximally Discriminative Facial Movement coding system (Max [14]), BabyFACS [20], and an adult pain metric [28,31] based on FACS Action Units [9,10]. NFCS pain scores were calculated by a certified NFCS coder based on presence or absence of 7 facial actions (brow bulge, eye squeeze, nasolabial furrow, open lips, horizontal stretch mouth, vertical stretch mouth, taut tongue), with higher scores indicative of higher pain on a scale from 0 to 7. Max scoring was done by a Max-certified coder based on emotion labels defined in the manual. BabyFACS interpretations were determined based on the manual by the creator of the measure. FACS AUs were coded based on the manual by a certified FACS coder. The Adult pain metric [28,31] was calculated based on the sum of the intensities of AU4 + AU6 or AU7 + AU9 or AU10 + AU43. All AUs are coded based on intensity (A to E, which translates to a score 0 to 5) except AU43, which is coded present or absent (0 or 1) based on the maximum intensity, AU 43E (eyes closed). Higher scores are indicative of higher pain and on a scale of 0 to 16. However, because scoring this pain metric for this study was calculated for a general category of a facial expression, intensity scores of AUs were based on the most common intensity scores within the category (C to D, which translates to a score of 3 or 4). Therefore, the possible range of scores was 0 to 13 [28,31].

3. Results

The 7 facial configurations identified are described in Table 2 and Fig. 1. Mouth descriptions (vertical and horizontal stretching) are based on the cry matrix described in the BabyFACS manual [20]. Analyses are described according to each facial expression (ie, color label), alongside a pictorial example.

Red (see Fig. 2): Overall average percentages of Red facial expression (collapsed over the minute post needle) were significantly different across age (χ^2 [2] = 34.58, $P < 0.001$; 2-month: 7.49%, 4-month: 5.12%, 6-month: 9.51%, and 12-month: 18.87%). Post hoc pairwise comparisons indicated a significant increase in Red facial expressions in infants from 2 to 12 months and from 4 to 12 months. Next, the trajectory of average percentage per 5-second increments over the minute for Red facial expressions was graphed separately for each age. In the first 30 seconds immediately after the last immunization needle, for each age there were increasing displays of Red facial expressions. In the 30 to 59 seconds following the last immunization needle, for each age there were decreasing displays of Red facial expressions over the minute. Despite their opposing direction, the amount of change during the first 30 seconds appears similar to that for the last 30 seconds. Finally, infants at 12 months displayed more Red facial expressions than at 2, 4, and 6 months across the entire minute.

Orange: The overall average percentage of Orange facial expressions occurred <5% of the minute following the needle, with no significant difference across age (χ^2 [2] = 6.61, $P = 0.09$; 2-month: 1.36%, 4-month: 3.07%, 6-month: 2.44% and 12-month: 2.37%). Overall, infants consistently displayed Orange facial expressions <5% of the time during every 5-second increment of the minute.

Yellow (see Fig. 3): The average percentage of Yellow facial expressions over the minute remained generally stable across age (χ^2 [2] = 10.09, $P = 0.02$; 2-month: 17.09%, 4-month: 10.36%, 6-month: 9.63%, and 12-month: 13.46%). In the first 15 seconds, for each age there were increasing displays of Yellow facial expressions. Infants at 12 months displayed the most Yellow facial expressions during the first 10 seconds. In the last 15 to 59 seconds, there was a decrease in the occurrence of Yellow facial expressions. During this later period, 2-month-olds continued to show high levels of Yellow expressions, while the levels dropped sharply at the 12-month appointment.

Green (see Fig. 4): There was a significant difference by age in overall mean occurrence over the minute (χ^2 [2] = 16.96, $P < 0.001$; 2-month: 2.49%, 4-month: 2.40%, 6-month: 2.51%, and 12-month: 0.00%), but no pairwise comparisons were significant.

Table 2
Descriptions of the categories of most common negative facial expressions.

Categories Distinguishing characteristics[a] Aus	Red Any cry mouth with oblique brows	Orange Horizontal cry mouth with eyes open	Yellow Big cry mouth (horizontal & vertical) with eyes open	Green Horizontal cry mouth with eyes closed	Blue Big cry mouth (horizontal & vertical) with eyes closed	Purple Any cry mouth without cheek raise or eyelid tightening	Black Non-cry negative mouth
1 + 3, 4	REQ	NO	NO	NO	NO	NO	OP
1 + 2 + 3, 4	NO	NO	OP	NO	OP	OP	NO
2 + 3, 2 + 4	NO	OP	OP	OP	OP	NO	OP
43	NO	NO	NO	REQ	REQ	OP	OP
6, 7	REQ	REQ	REQ	REQ	REQ	NO	OP
20	REQ	REQ	REQ	REQ	REQ	REQ	NO
25 + 26ab	OP	OP	NO	OP	NO	OP	OP
25 + 26c/27	OP	NO	REQ	NO	REQ	OP	OP
17	OP	OP	NO	OP	OP	OP	OP

REQ, required; OP, optional; NO, not allowed.
[a] Detailed verbal descriptions of categories can be found in Table 3.

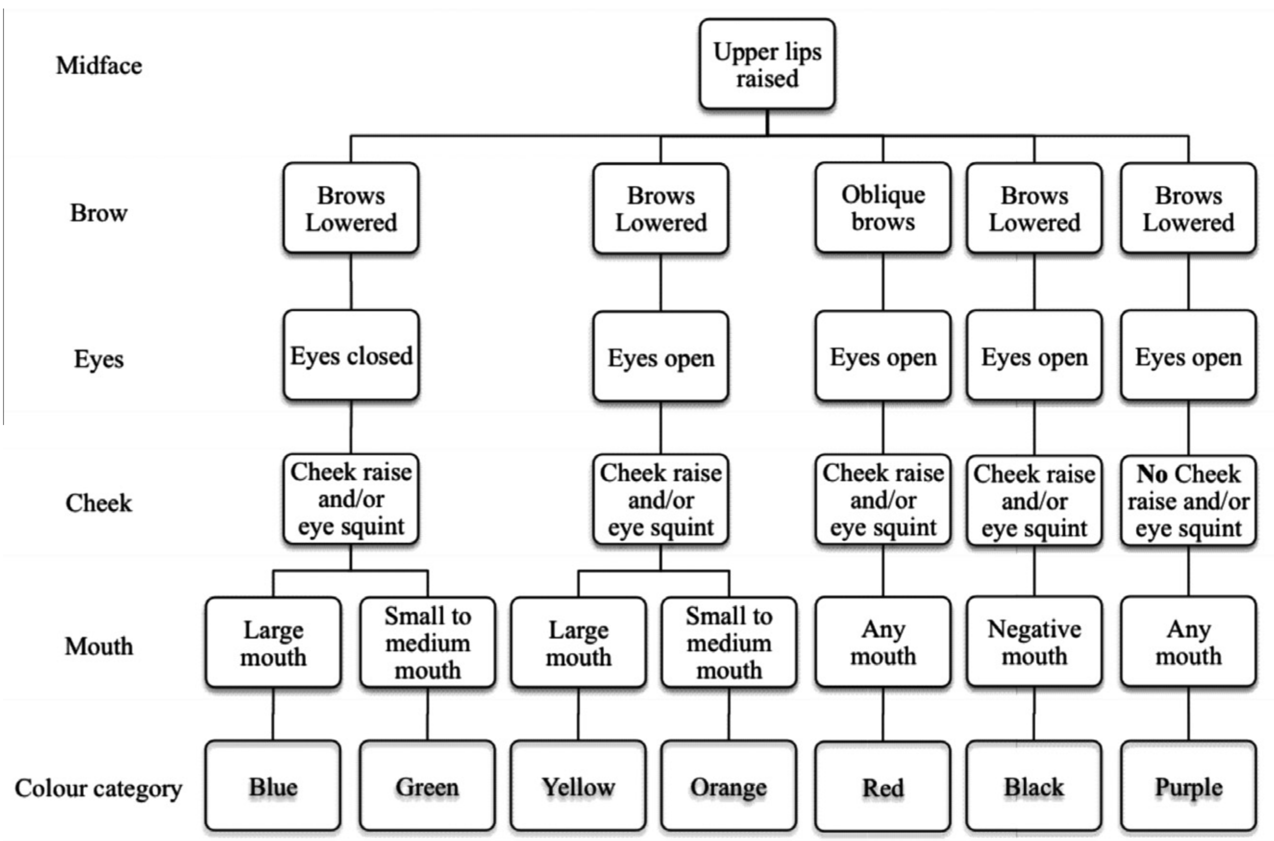

Fig. 1. Simplified schematic representation of the categorization of facial expressions.

At 12 months, infants did not display Green facial expressions in response to the last immunization needle. For infants at 2, 4, and 6 months, Green facial expressions occur within the first 10 seconds after immunization needle, then diminish until the end of the 1-minute epoch.

Blue (see Fig. 5): Blue facial expressions significantly differed in mean occurrence over the year (χ^2 [2] = 81.13, $P < 0.001$; 2-month: 25.70%, 4-month: 13.50%, 6-month: 9.85%, and 12-month: 3.56%). Post hoc tests revealed significant decreases in the occurrence of Blue facial expressions over age, with 2-month-old infants displaying significantly more Blue expressions than 4-, 6-, and 12-month olds and 12-month olds displaying significantly fewer Blue expressions than 4- and 6-month olds. At all ages, infants displayed the most Blue facial expressions within the first 5 seconds after the immunization needle and decreased steadily within the first 15 seconds. Consistently over the minute, infants at 2 months displayed the most Blue facial expressions, followed by infants at 4, 6, and 12 months. However, over the last 40 seconds, the 4-, 6-, and 12-month trajectories appeared indistinguishable.

Purple: There were no age differences in average occurrence of Purple facial expressions (χ^2 [2] = 5.83, $P = 0.12$; 2-month: 1.40%, 4-month: 1.76%, 6-month: 1.87%, and 12-month: 1.93%). The amount of Purple facial expressions remained low and flat across the postimmunization minute for each age. Overall, infants consistently displayed Purple facial expressions <2% of the time after the immunization needle.

Black (see Fig. 6): Black facial expressions significantly differed in mean occurrence across age (χ^2 [2] = 25.42, $P < 0.001$; 2-month: 4.26%, 4-month: 3.20%, 6-month: 3.60%, and 12-month: 11.24%). Post hoc pairwise comparisons revealed significant increases in

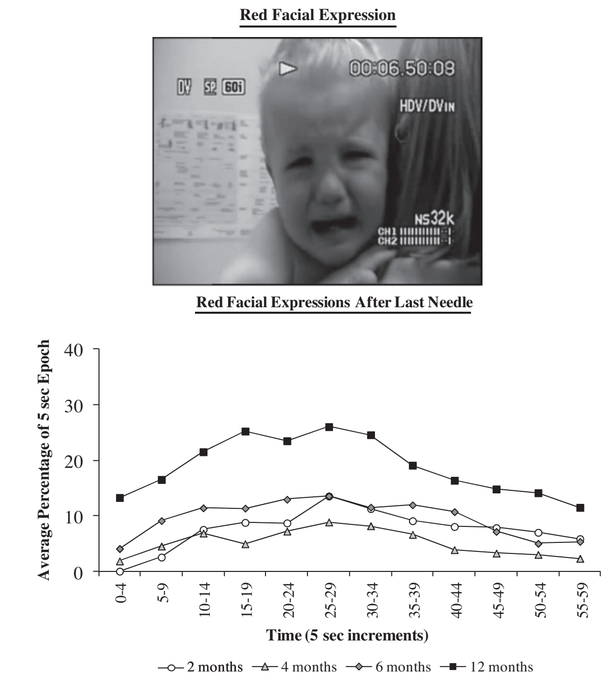

Fig. 2. Example of a Red facial expression and graph presenting the proportion of Red facial expressions displayed in response to the last needle.

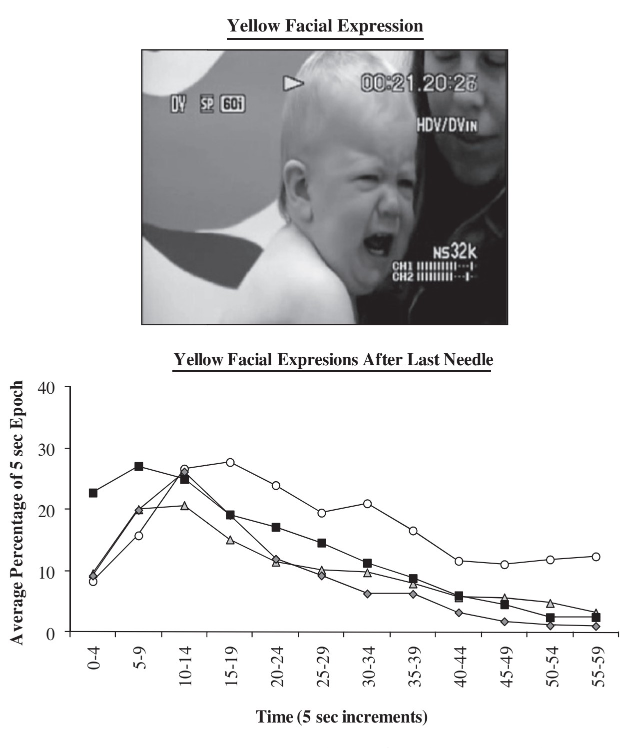

Fig. 3. Example of a Yellow facial expression and graph presenting the proportion of Yellow facial expressions displayed in response to the last needle.

Black facial expressions from 2, 4, and 6 months to 12 months. Lastly, for each age, Black facial expressions had a slight tendency to increase over the 1 minute following the immunization needle. Infants at 12 months displayed consistently more Black facial expressions across the entire minute compared to the younger ages.

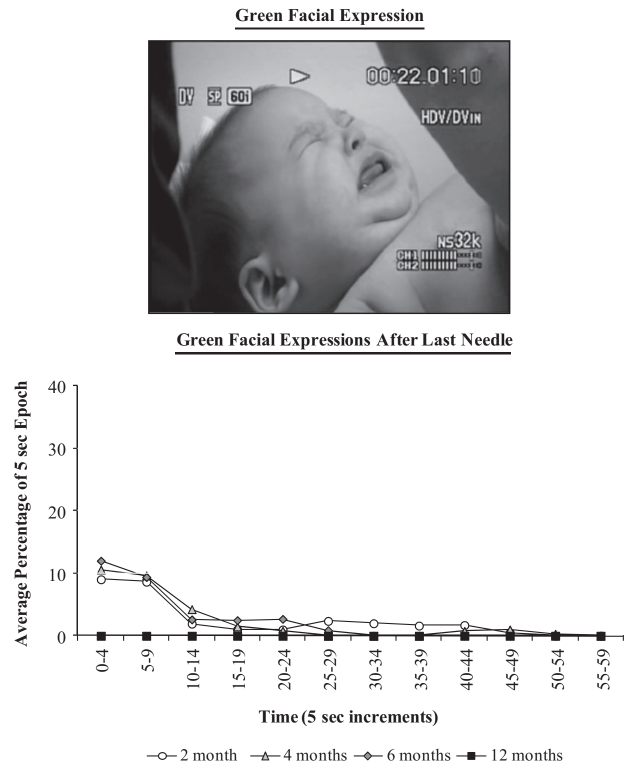

Fig. 4. Example of a Green facial expression and graph presenting the proportion of Green facial expressions displayed in response to the last needle.

4. Discussion

Infants' facial actions were comprehensively coded following an acutely painful procedure until 1 minute after a noxious stimulus (ie, immunization). Many of the categories of facial expressions empirically identified in this study involved some of the same components as the adult pain expressions (ie, Red, Orange, Yellow, Green, and Blue expressions). In fact, most of the variation in adult facial expressions in response to pain can be limited to 4 facial actions [28]: brow lowering (AU4), cheek raising/eyelid tightening (AU6/AU7), nose wrinkling/lip raising (AU9/AU10), and eye closure (AU43). As with the adult facial expression of pain [28], the degree of vertical mouth opening (AU26/AU27) was not a sensitive or specific component of the infant facial expression of pain. However, a distinct difference emerged between expressions of pain in adults and infants. Specifically, a horizontal stretching of the mouth (AU20) has not always been associated with expressions of pain in adults [28,31], but this study suggests that it is an important component of the infant facial expression of pain.

Horizontal stretching of the mouth is likely more integral to infant facial expressions of pain because, along with mouth opening, it is the principal component of cry faces and is usually associated with cry vocalizations. Adults have more control over their pain responses and rarely cry in response to an acute pain stimulus such as an immunization. Most infants, however, cry in response to those very same stimuli. More generally, crying is the first form of vocal communication exhibited by infants after birth, and the most common response to generalized distress or discomfort. Therefore, the intensity of horizontal stretching of the mouth may be considered when assessing pain intensity in infant facial expressions. This idea is supported by work in the infant pain literature [11,12].

In this study we examined the facial expressions of pain among infants at 4 different ages in a systematic fashion. This study went further than earlier studies because we coded the entire spectrum

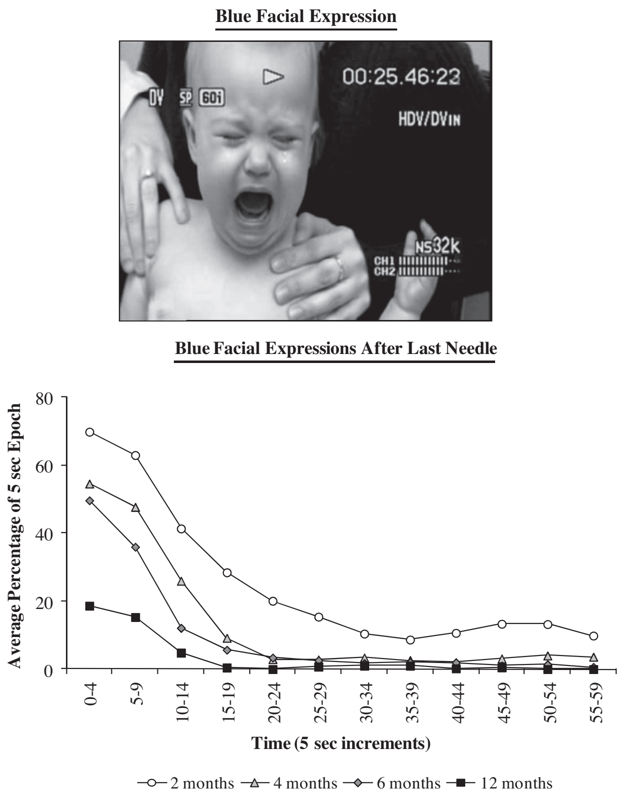

Fig. 5. Example of a Blue facial expression and graph presenting the proportion of Blue facial expressions displayed in response to the last needle.

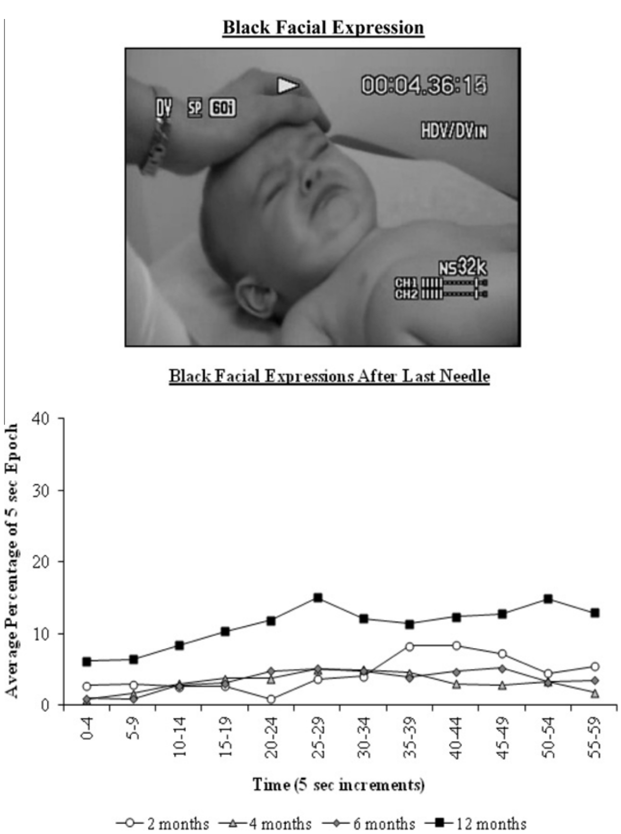

Fig. 6. Example of a Black facial expression and graph presenting the proportion of Black facial expressions displayed in response to the last needle.

of facial actions, not just those presumed to be associated with pain. NFCS [11,12], a measure of pain, has been shown to lack the ability to differentiate between pain-related and non-pain-related distress [1]. One challenge in defining differing emotional expressions, regardless of age, is the overlap of facial actions involved in all negative emotional expressions. Past studies with infants [2,16,17] ignored this commonality in their attempts to code discrete negative emotions in infants' distress responses. However, in pain contexts, this overlap makes tremendous sense. An influential review regarding the affective dimension of pain [27] describes a complex, simultaneous affective reaction to pain rather than a unitary response. The findings herein demonstrate that in the first year of life, there is a core set of facial actions in response to pain and regulation of distress from pain. This study has further established that this core set has 7 readily discernible constellations. We speculate that, owing to a common core set of facial actions, these 7 constellations do not reflect discrete negative emotions but rather, different variants and intensities of sensory or affective pain responses to noxious stimuli.

Although we coded every visible facial muscle action using a fine-grained, anatomically based coding system, we did not observe expressions of discrete negative emotions in the immediate postneedle epoch. Table 3 illustrates the facial expressions identified in this study and their suggested interpretations according to the BabyFACS [20], NFCS [11,12], and Max [14] manuals, as well as an intensity metric for adult pain [28] specified in terms of FACS AUs [9,10]. As seen in Table 3, the constellations of facial expressions shown by infants immediately following immunization do not correspond to prototypical, adult-like expressions of discrete negative emotions. Instead, Table 3 demonstrates that according to NFCS, BabyFACS, and the adult pain metric, all faces would be classified as distress or pain. However, according to the Max system, only Blue would be classified as discomfort-pain. Yellow would be coded as discrete anger, and other configurations would be coded as blends of different negative emotions. It is important to note that the Max formulas for discrete negative emotions are simply based on how widely open an infant's eyes and mouth are. While it is feasible to suggest that the degree of mouth and eye openness reflects discrete emotions, these distinctions have not been supported by empirical research [6,21,22]. We suggest that these variations in the intensities of distress configurations may reflect sensory intensity differences and developing abilities to regulate distress.

Earlier, we suggested that the 7 constellations identified here reflect different pain variants. From what is generally known about the immunization experience and acute procedural pain, pain is felt the most intensely initially (ie, from the skin breakage and the vaccine being pushed into the skin or muscle) and gradually abates as a function of time [23,26]. Thus, it appears logical that facial expressions closest in time to the needle may be more representative of the most intense pain expression (high noxious sensory stimulation with initial reactivity), while facial expressions closer to 1 minute post needle may reflect blended expressions of negative emotions and less intense sensory pain, as well as efforts to regulate pain and distress.

Our findings are consistent with research demonstrating that infants do not show differentiated facial expressions of discrete negative emotions like sadness and fear in the first year of life [6,21] in response to pain. It also shows that there is not a single, stereotyped pain expression during infancy. Infant facial expressions serve the purpose of nonverbally communicating a need or a desire [24,25]. When young infants experience pain (and negative emotion associated with pain), they need to communicate that they need help. A generalized distress expression serves a strong evolutionary function in that it would likely result in proximity from a caregiver [18]. As gaining proximity or help from a caregiver

Table 3
Descriptions and interpretations of the categories of negative expressions.

Category name	Description of expression	NFCS[a]	Max[b]	BabyFACS[c]	Adult pain[d]
Red	- Brow oblique and drawn together - Cheek raise and/or eyelid tightening - Lip raise or nasolabial furrow - Slightly to moderately open mouth - Horizontal stretch of the mouth	- Pain score: 3-5	- Sadness/anger blend - Sadness/fear blend	- Pre-cry or cry face - Fuss face - Moderate distress	- Pain score:[d] 9-12
Orange	- Brow knitting and/or knotting - Cheek raise and/or eyelid tightening - Lip raise or nasolabial furrow - Slightly open mouth - Horizontal stretch of the mouth	- Pain score: 4-5	Anger/fear blend	- Grimace - Fuss face - Mild to moderate distress	- Pain score:[d] 9-12
Yellow	- Brow knitting and/or knotting - Cheek raise and/or eyelid tightening - Lip raise or nasolabial furrow - Widely open mouth - Intense horizontal stretch of the mouth	- Pain score: 5-6	- Anger	- Intense cry face - Intense distress	- Pain score:[d] 9-12
Green	- Brow knitting and/or knotting - Cheek raise and/or eyelid tightening - Eyes closed - Lip raise or nasolabial furrow - Slightly open mouth - Horizontal stretch of the mouth	- Pain score: 5-6	- Discomfort-pain/fear blend	- Moderate cry face - Fuss face - Moderate distress	- Pain score:[d] 10-13
Blue	- Brow knitting and/or knotting - Cheek raise and/or eyelid tightening - Eyes closed - Lip raise or nasolabial furrow - Widely open mouth - Intense horizontal stretch of the mouth	- Pain score: 6-7	- Discomfort-pain	- Intense cry face - Intense distress	- Pain score:[d] 10-13
Purple	- Brow knitting and/or knotting - Lip raise or nasolabial furrow - Slightly to moderately open mouth - Horizontal stretch of the mouth	- Pain score: 3	- Partial anger	- Mild cry face - Fussy	- Pain score:[d] 6-8
Black	- Brow knitting and/or knotting - Lip raise or nasolabial furrow - Lip corners may be depressed - Possible pout or "horseshoe" mouth	- Pain score: 2-3	- Anger/sadness blend - Discomfort-pain/sadness blend	- Pouts - Horseshoe-mouth - Emotion regulation - Modulated distress	- Pain score:[d] 6-8

[a] Neonatal Facial Coding System (NFCS) manual [11,12].
[b] Maximally Discriminative Facial Movement coding system (Max) manual [14].
[c] Facial Action Coding System for Infants and Young Children (BabyFACS) manual [20].
[d] Prkachin's [28,31] adult pain intensity metric based on Facial Action Coding System (FACS) Action Units [9,10].

quickly is the likely end goal for infant pain communication, it seems crucial for a caregiver to discern exactly why the young infant needs help and more efficient to simply be signaled that help is needed urgently and that proximity is necessary. Consistent with previous ethological thinking and an ontogenetic perspective, we agree that a generalized infant cry is enough to engage the caregiver system regardless of the specific cause [18].

By tracking the occurrence of infants' facial expressions over the first minute post needle and the decrease of acute pain responding in infancy over the first year of life, we can attempt to speculate about the communicative goals of the facial expressions discerned and how they may change as the infant ages. The goal of this study was to provide the field with a broad inventory of all possible infant facial muscle configurations that occur in an acute pain context. We aimed to gain novel data germane to understanding the development and regulation of negative affect over the first year of life. Our analysis of the temporal trajectories of the 7 categories of facial responses we identified suggested that these are pain-related distress expressions ranging from the most intense pain and sensory overload immediately following inoculation (Blue expressions with tightly closed eyes: "complete sensory overwhelm") to decreasing degrees of pain (Green, with closed eyes and less intense cry mouth: "moderate sensory overwhelm"), and increasing capacity with time and age to regulate distress (Red, Orange, and Yellow expressions). The Yellow expressions, with a big cry mouth and open eyes, may reflect attempts to regulate intense distress and maintain visual contact ("regulation from intense distress"); Red expressions may be labeled "regulation from moderate distress;" and Orange expressions may be labeled "regulation from mild/moderate distress." Lastly, facial configurations with less intense cry face components might be labeled "mild distress" (Purple) or "mild discomfort" (Black). We propose that these 7 categories of expressions may have evolved to allow infants to communicate 2 crucial broad states to caregivers: level of distress and degree of regulation from distress. Although these categories of facial expressions are visually distinguishable within the context of pain, we suggest that they do not represent expressions of discrete negative emotions.

4.1. Study limitations

Infants did not receive the same immunization at 12 months as they did at 2, 4, and 6 months [32]. However, regardless of vaccine protocol, all infants were exposed to a highly painful acute stimulus and showed modulation of the intensity of distress expressions over the minute. Nevertheless, following infants longitudinally at 2, 4, 6, and 12 months provided evidence of developmental changes in the relative frequency of the different expressions observed and their time course.

Our analysis of expressions was limited to facial expressions. Caregivers almost always have access to vocalizations and body movements when judging an infant's emotions; therefore, there is strong merit in considering these expressive modalities in relation to facial expressions. However, facial expressions collectively are used by infants, children, and adults to communicate the infants' emotions and behavioral dispositions to others [3,8,21] and are therefore critical in their own right.

Since our analyses focused on responses to pain within the first minute post needle, we cannot draw any general conclusions outside this specific context. However, our findings are consistent with those of studies that have emphasized the importance of infants' facial expressions for parents' ability to read and respond sensitively to their infants' distress [18,21,23].

Conflict of interest statement

The authors have no financial interest in the results of this research. None of the authors have any conflict of interest with this work.

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