DEVELOPMENT OF CARDIOVASCULAR INDICES OF ACUTE PAIN RESPONDING IN INFANTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Cardiovascular indices of pain are pervasive in the hospital setting. However, no research has examined the development of cardiac responses to acutely painful procedures in the first year of life. Our main goal was to synthesize evidence regarding the development of cardiovascular responses to acutely painful medical procedures over the first year of life in preterm and term born infants. A systematic search retrieved 6994 articles to review against inclusion criteria. A total of 41 studies were included in the review. In response to acutely painful procedures, most infants had an increase in mean heart rate (HR) that varied in magnitude both across and within gestational and postnatal ages. Research in the area of HR variability has been inconsistent, limiting conclusions. Accordingly, longitudinal research is needed to further understand the inherent variability of cardiovascular pain responses across and within gestational and postnatal ages, and the causes for the variability.

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1. Introduction

Although skepticism towards infant pain characterized much of the 20th century research and clinical practices (Rodkey & Pillai Riddell, 2013), it is now well established that infants' pain transmission pathways in the brain are fully developed by 22 to 24 weeks gestation (Schwaller & Fitzgerald, 2014). Conversely, pain inhibitory systems are not fully developed in infants, suggesting that infants may feel even more pain than older children (Schwaller & Fitzgerald, 2014). Owing to a variety of caregiver, infant and health care practitioner factors (see Mitchell, Brooks, & Roane, 2000), emphasis on the assessment and management of infant pain is lacking.

Improper management of acute pain has been associated with various short- and long-term negative physiological and psychological consequences. Specifically, increased metabolic rate during painful experiences has been associated with short-term consequences such as exacerbating injury, increased potential for chronic pain, delayed wound healing, increased risk of infection, and alterations in pain sensitivity (Denk, McMahon, & Tracey, 2014; Grunau, 2013; Kristjansdottir et al., 2012). Additionally, long lasting consequences include delays in motor and brain development, as well as deficits in cognition and emotion regulation (Brummelte et al., 2012; Grunau et al., 2009; Ranger et al., 2013; Valeri, Holsti, & Linhares, 2015; Vinall et al., 2013; Vinall et al., 2014). Therefore, it is important to establish empirically-based behavioural and physiological pain assessment tools that can be utilized in infancy to begin the pain management process.

The major challenge with infant pain assessment is that neonates cannot self-report their subjective experience of pain, and there is a lack of agreement on the best modality of assessing infant pain, whether it be a cortical, biochemical, physiological, or behavioural measure

(Rouzan, 2001). Moreover, recent work has not only suggested discordance among (Slater et al., 2008, Slater et al., 2010), but also within, assessment modalities (Pillai Riddell et al. 2013). For example, the validity and reliability of physiological measures of infant pain are presently disputed, due to these measures being influenced by additional physiological confounders (e.g., infection, respiratory rate) (Maxwell, Malavolta, & Fraga, 2013).

Despite the abovementioned disputes, cardio-physiological indices of pain, such as heart rate (HR), and HR variability (HRV), are pervasive in the hospital setting (Grunau et al., 2006). Indeed, cardiac measures are well-established noninvasive proxies of cardiac autonomic control, and have been integrated in well-established pain assessment tools for preterm and term born infants, as well as young children (Ambuel, Hamlett, Marx, & Blumer, 1992; Cignacco, Mueller, Hamers, & Gessler, 2004; Gibbins et al., 2014; Hummel, Lawlor-Klean, & Weiss, 2010; Stevens et al. 2014; Stevens, Johnston, Petryshen, & Taddio, 1996; van Dijk et al. 2000). However, despite this proliferation, there appears to be no research that has longitudinally examined the development of cardiac responses to acutely painful procedures in either preterm or term born infants. Thus, it is currently unknown how physiological pain regulation develops across the first year of life in term born and preterm infants. It is important to better understand the systems controlling cardiovascular function, as they are closely coupled with the perception of pain (Faye et al., 2010; Bouza 2009).

The typical developmental pattern of behavioural pain responding suggests that it is variable across the first year of life in term born infants (Pillai Riddell et al., 2013). This behavioural work demonstrates inherent limits to the validity of averaging over developmental stages within infancy (Pillai Riddell et al., 2013). Complicating this picture further, it is important to note that the nervous system develops differently in those born at varying levels of

prematurity (e.g., Craig et al., 1993; Grunau et al., 2010; Johnston & Stevens, 1996; Singh et al., 2000), and there is a significant trajectory of cortical, physiological and behavioural development over the first years of life (e.g., Chatow, Davidson, Reichman, & Akselrod, 1995; Sugihara, Allan, Sobel, & Allan, 1996). Thus, it is crucial to take a more detailed and developmental approach to other measurement modalities within the field of infant pain. The purpose of this systematic review and meta-analysis is to synthesize existing evidence on the development of cardiovascular responses to acutely painful medical procedures over the first year of life in both preterm and term born infants.

2. Method

2.1. Search strategy

With the assistance of an academic librarian at the University of Toronto, a systematic search was conducted in Medline, Embase, PsychINFO, and CINAHL in July 2014 for English-language references. Searches were limited to articles published from 1970 to 2014 in order to encompass historical and contemporary articles and reviews. Search terms related to acute pain procedures, cardiovascular measures, and infants (0-3 years of age) were systematically paired (See Appendix A). We also hand searched reference lists of relevant studies and systematic reviews on cardiovascular responses to acute pain in infants. Our review followed an a priori protocol according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009). The review protocol was registered on the PROSPERO website before data extraction (registration no. CRD42015016398) (Booth, 2013).

2.2. Inclusion and exclusion criteria and study selection

We included prospective observational or descriptive studies of individuals equal to or

under 3 years of age undergoing an acutely painful procedure, which was monitored using a cardiovascular measure. Our definition of observational studies included cohort studies in which participants were prospectively identified and followed up during acutely painful procedures using cardiovascular indices, as well as cross-sectional studies that observed an acutely painful procedure using a cardiovascular measure across different gestational or postnatal ages. We also included control group data from pain manipulation studies, and prospective randomized or randomized controlled trials (RCTs) that investigated the effectiveness of pain management strategies using cardiovascular measures.

Studies were excluded if they described non-human animal models of pain, did not measure an acutely painful event nor included a cardiovascular measure of acute pain, were prospective randomized, RCTs, or pain manipulations that did not include a control group, were review articles, case studies, or conference abstracts, or studies that included participants that differed in age at measurement (i.e., collapsing over one or more months) or gestational age (GA) (i.e., collapsing across at least four months GA). Of note, most studies that were discarded for collapsing over age of measurement were averaging over age spans within infancy greater than 6 months.

Two authors designed the abstract selection criteria with an initial selection of 500 abstracts (J.W. and R.P.R.). Three authors (A.P., J.W., and P.T.) independently read and selected from all the retrieved references and abstracts. Any disagreements between reviewers were resolved through discussion. The percent agreement between the raters ranged from .96 to 1.0. Full texts of potentially eligible studies were retrieved (See Figure 1).

2.3 Data extraction and quality assessment

A database was created recording GA at birth, postnatal age at measurement, a description of the cardiovascular results, and any covariates that were included when analyzing whether there were differences in cardiovascular measures following an acutely painful medical procedure. It was important to investigate covariates included in the studies, as there are a number of physiological and behavioural variables known to affect the cardiovascular system (Maxwell, Malavolta, & Fraga, 2013). We reasoned that delving into what variables were controlled for might help explain why there is variability in cardiovascular measures. Where information was incomplete, the authors were contacted by email.

Due to a gold-standard quality assessment measure not being available for observational studies (see meta-analysis by Sanderson, Tatt, & Higgins, 2007), a modified checklist combining Downs and Black (1998) and Crombie (1996) was utilized (See Appendix B). These measures were chosen based on a multidisciplinary collaborative review in the field discussing quality in case-control, cohort, and cross-sectional studies (Sanderson et al 2007). Fifty percent of the extractions were consensus coded for quality scores to ensure reliability. Disagreements were minimal and were resolved through discussion, to obtain a final score for each paper. Criteria were scored as "Yes" (1), "No" (0), or "Unable to Determine." Positively scored criteria were added up in order to obtain a total quality score for the paper. The maximum obtainable score for the paper was 20 for cross-sectional studies and 21 points for cohort studies. The results were expressed as percentages of the total obtainable score.

2.4. Analysis

We aimed to synthesize evidence on the development of cardiovascular responses to acutely painful procedures in preterm and term born infants. For qualitative analysis, group-

specific data were first separated by age at measurement, and subsequently subdivided by GA at birth, as well as cardiovascular outcome measures (i.e. mean heart rate [HR], HR change, maximum HR, low frequency heart rate variability [LF HRV], high frequency heart rate variability [HF HRV], low frequency/high frequency ratio [LF/HF ratio]).

When possible, studies that included mean HR, HR change, maximum HR, LF HRV, HF HRV, and/or LF/HF ratio and the standard deviations were then quantitatively analyzed using the MEANS procedure in SAS, which computed descriptive statistics for variables within the aforementioned GA groups during specific postnatal ages.

3. Results

3.1. Studies included

We identified 6994 articles from the electronic searches after removal of duplicates. These articles were then reviewed by title and abstract, and were included or excluded based on *a priori* selection criteria. A total of 180 articles were then reviewed by full-text review, and of these, 41 articles (involving 1552 participants) fulfilled the inclusion criteria (Abad et al., 2001; Altun-Koroglu et al., 2010; Bilgen et al., 2001; Bucher et al., 2000; Campos et al., 1994; Cong et al., 2009; Cong et al., 2012; Craig et al., 1993; de Jesus et al., 2011; de Oliveira, 2012; Gormally et al., 2000; Goubet et al., 2001; Gray et al., 2000; Greenberg et al., 2002; Grunau et al., 2010; Haouari et al., 1995; Jatana et al., 2003; Johnston et al., 2007; Johnston et al., 2006; Kostandy et al., 2013; Leite et al., 2009; Lindh et al., 2000; Lindh et al., 2003; Lucas-Thompson et al., 2008; Oberlander et al., 2002; Oberlander et al., 2002; Oberlander et al., 2005; Ors et al., 1999; Owens 1984; Sajedi et al 2006; Shibata et al., 2012; Singh et al., 2000; Stevens et al., 1993; Stevens et al., 1994; Taksande et al., 2005; Upadhyay et al., 2004; Uyan et al., 2005; Walden et al., 2001;

Weissman et al., 2009; Weissman et al., 2012). These studies underwent quality assessment and data extraction and were included in the final review.

3.2. Study characteristics

Table 1 provides a detailed overview of the studies included, including sample size, country of origin, GA at birth, postnatal age at measurement, acutely painful procedure, cardiovascular measure, study design, and quality assessment score.

Generally speaking, a quarter of the studies were from Canada, a quarter from the United States and a quarter from Europe, with the remaining studies coming from Asia, the Middle East and Brazil. The majority of studies were randomized trials, and encompassed infants born between 24 to 42 weeks GA, that were tested between postnatal day 1 and postnatal month 4. The most common acutely painful procedure that was utilized in the studies was heel stick, and mean HR was the most frequently used cardiovascular measure. In terms of the range of quality scores for the papers, the lowest score was 40% (Singh et al., 2000), the median quality score for the papers was 75%, and the highest score was 86% (Walden et al., 2001).

Age categorizations were difficult to obtain due to the variability between studies in the age groups they analyzed. Based on the available data, the results will be organized by the following postnatal ages (i.e. age at measurement): 7 postnatal days or less, 1 to 2 postnatal weeks, 3 postnatal weeks, and 1, 2, 3, and 4 postnatal months. Due to the majority of data being published on infants within the first 7 postnatal days, tables will only be presented for these studies (See Tables 3 to 10). In addition, within each age at measurement category, results will then be subdivided by accepted categorizations of GAs (Blencowe et al., 2010) and the cardiovascular measures examined. Due to the large variability in choice of covariates only the

presence of covariates will be noted, with a comprehensive list being provided in Table 2 across studies.

3.3. Age at Measurement: Seven Postnatal Days or Less

3.3.1. Extremely preterm

25 to 27 Weeks GA.

Mean Heart Rate: One high quality study used mean HR to describe the acute pain experience following a heel stick in those born at 25-27 weeks GA and were measured at 5.50 postnatal days (Craig et al., 1993). The authors found that that those born at 25-27 weeks GA did not have a significant increase in mean HR in response to a heel stick in the first week of life. The study found that the mean HR was 172.38 bpm in response to heel stick.

3.3.2. Very preterm

28 to 32 Weeks GA.

Mean Heart Rate: A total of 3 studies investigated the mean HR response to heel stick in those born at 28 to 32 weeks GA and measured at 3 to 6 postnatal days (Cong et al., 2009; Craig et al., 1993; Lucas-Thompson et al., 2008). The three studies found that heart rate significantly increased following the heel stick (Cong et al. 2009; Craig et al., 1993; Lucas-Thompson et al., 2008). The studies found that mean HR post-acute pain ranged from 155.25 to 169.27 bpm (See Table 3). The variability may be due to only one study including covariates (i.e. number of prior heel sticks, duration of blood draws, sex, baseline heart rate) in their analysis of the cardiovascular measures (Lucas-Thompson et al., 2008). Overall, the studies were generally high quality.

Heart Rate Change: One lower quality study examined mean HR change in response to heel stick at 4 postnatal days (Goubet et al., 2001), and found that HR was significantly higher during blood collection compared to baseline HR (See Table 4).

Heart Rate Variability: One high quality study investigated LF and HF HRV, as well as the LF/HF ratio in response to heel stick at 6 postnatal days (Cong et al., 2009). The authors found that LF and HF HRV increased in response to heel stick (See Table 5 and 6, respectively), while the LF/HF ratio decreased in response to heel stick (See Table 7).

3.3.3. Moderate to late preterm

32 to 34 Weeks GA.

*Mean Heart Rate**:*** A total of 4 studies investigated the mean HR response to heel stick (Johnston & Stevens, 1996; Lucas-Thompson et al., 2008; Stevens et al., 1993; Stevens et al., 1994), while 1 study investigated the mean HR response to venipuncture (Singh et al., 2000). All studies investigated infants between 3 and 7 postnatal days. Overall, mean HR was found to increase in response to acute pain. However, the magnitude of responses was variable and ranged from 154-183.4 bpm, with mean HR being higher in the study using venipuncture as the acutely painful stimulus. Additionally, variability in the magnitude of mean HR response may be due to over half of the studies not including covariates in their analysis (Singh et al., 2000; Stevens et al., 2003; Stevens et al., 1994). In the two studies that did include covariates, the authors controlled for the frequency of invasive procedures, severity of illness, ventilation status, sex, number of prior heel sticks, duration of blood draws, and baseline heart rate. Overall, the quality of the studies varied (i.e. 40% compared to 85%).

Heart Rate Variability: One high quality study investigated mean total HRV in response to heel stick at 5 postnatal days or less (Stevens et al., 1994). It was found that total HRV was

not significantly different in response to heel stick (See Table 8). However, total HRV represented the standard deviation of the mean HR, which may have affected the accuracy of the measure.

34 to 37 Weeks GA.

Mean Heart Rate: A total of 2 studies investigated mean HR response to heel stick (Craig et al., 1993) or venipuncture (Singh et al., 2000). The studies investigated infants at 3 to 7 postnatal days. In both studies, mean HR increased following the acute pain procedure. Mean HR in response to acute pain was found to be 165.3 and 163.2 bpm after heel stick and venipuncture, respectively. The quality of the studies was found to vary (i.e. 40% compared to 85%).

3.3.4. Full term

Mean Heart Rate: A total of 9 studies investigated mean HR response to heel stick (Campos et al., 1994; Craig et al., 1993; Gormally et al., 2000; Gray et al., 2000; Leite et al., 2009; Lindh et al., 1999; Oberlander et al., 2002; Shibata et al., 2000; Weissman et al., 2012), 4 in response to venipuncture (Abad et al., 2001; Lindh et al., 2000; Singh et al., 2000; Taksande et al., 2005), and 1 in response to vaccination (Kostandy et al., 2013). The studies investigated infants at 0 to 7 postnatal days. Overall, mean HR increased post acute pain procedure; however, as in premature infants, the magnitude of the response was variable in term born infants. Mean HR ranged from 134 to 174 bpm in response to acute pain. Out of the14 studies investigating mean HR response to acute pain, only three included covariates in their analyses (Campos et al., 1994; Gormally et al., 2000; Oberlander et al., 2002), which again may explain the variability in the results. These studies included the number of additional sticks required to obtain the blood sample, duration of the heel stick, frequency of crying, average HR, pre-intervention baseline

(percentage of time crying in the last two minutes before beginning the interventions), breast-fed (yes/no), SSRI exposure (yes/no), age at time of acute pain, maternal analgesia (yes/no), dose of SSRI at time of delivery, and dose of clonazepam at time of delivery. Overall, the quality of the studies was variable and ranged from low to high quality.

Maximum Heart Rate: A total of 6 studies investigated maximum HR while infants underwent a heel stick procedure at 2 to 7 postnatal days (Altun-Koroglu et al., 2010; Bilgen et al., 2001; Campos et al., 1994; de Jesus et al., 2011; Owens 1984; Uyan et al., 2005). Overall, maximum HR was found to increase in response to the heel stick, and ranged from 149 to 196 bpm (Table 9). Two studies included covariates in their analysis (Campos et al., 1994; de Jesus et al., 2011), which were the number of additional sticks required to obtain the blood sample, the duration of the heel stick, the frequency of crying, the average HR, gestational age, birth weight, sex, mode of delivery, diabetic mother (yes/no), breastfed one hour before puncture (yes/no), or received oral glucose (yes/no). Overall, the studies were relatively lower in quality (i.e. 60 to 75%).

Heart Rate Change: A total of 9 studies investigated mean HR change in response to heel stick (Bucher et al., 2000; Gray et al., 2000; Haoari et al., 1995; Jatana et al., 2003; Ors et al., 1994; Owens, 1984; Uyan et al., 2005; Weissman et al., 2009) and intramuscular injection (Sajedi et al., 2006) from 0 to 7 postnatal days. In all studies, mean HR increased significantly in response to acute pain. Mean HR was found to increase by 31 to 49 bpm, or between 11 to 38 percent. Only three studies included covariates in their analysis of the cardiovascular measure (Bucher et al., 2000; Owens 1984; Sajedi et al., 2006), which included sex, nurse, number of lances needed, baseline heart rate, and activity. The studies included were generally high in quality.

Heart Rate Variability: A total of 8 studies investigated mean HF HRV, 6 studies investigated LF HRV, and 3 studies investigated the LF/HF ratio or total HRV during heel stick (de Oliveria et al., 2012; Gormally et al., 2000; Greenberg et al., 2002; Lindh et al., 2009; Oberlander et al., 2002; Weissman et al., 2009, Weissman et al., 2012) or venipuncture (Lindh et al., 2000). Total HRV was found to be variable in the two studies, with one study suggesting it increases in response to heel stick (Lindh et al., 1999), and the other study suggesting it decreases in response to venipuncture (Lindh et al., 2000). It is possible that the two acutely painful procedures may have differed in the amount of pain caused. There was also variability in HF HRV, with some studies finding HF HRV decreased in response to acute pain (de Oliveira et al. 2012; Greenberg et al., 2009; Oberlander et al., 2002; Weissman et al., 2009, 2012), and some studies finding no difference in HF HRV in response to acute pain (Gormally et al., 2000; Lindh et al., 1999; Lindh et al., 2000). These differences in response patterns may be due in part to the heterogeneity of covariates included in four of the studies (de Oliveira et al., 2012; Gormally et al., 2000; Greenberg et al., 2009; Oberlander 2002), and the lack of covariates included in the four remaining studies (Lindh et al., 1999; Lindh et al., 2000; Weissman et al., 2009; Weissman et al., 2012). LF HRV was found to decrease in response to acute pain in four studies (Lindh et al., 2000; Oberlander 2002; Weissman et al., 2009, 2012) and increase in one study (Lindh et al., 1999). Only one study included covariates in their analyses, which may help to explain the variability in the results (Oberlander 2002). Finally, the LF/HF ratio was found to increase in the three studies (Oberlander et al., 2002; Weissman et al., 2009; Weissman et al., 2012), with only one study including covariates in the analysis (Oberlander et al., 2002). These studies included infants between 0 and 7 postnatal days, and the studies ranged in quality from 50 to 80%.

3.3.5. Quantitative analysis of mean HR

Due to the variability in measurement strategies across studies, only mean HR was amenable to meta-analysis. Separate pooled averages were created to specifically examine mean HR differences based on GA at birth in those born at 28-32, 32-34, 34-37, and 37-42 weeks GA. Based on the confidence intervals overlapping in all GA groups, the results suggest that pooled mean HR responses to acute pain did not statistically differ based on GA at birth (Table 10). However, based on certain multimodal pain scales (e.g., PIPP-R; Stevens et al., 2014), full term and preterm infants would be in separate score categories for pain, which illustrates clinically significant differences in mean HR response to acute pain depending on GA at birth.

3.3.6. Summary

Mean Heart Rate: The magnitude of cardiovascular response was variable across GAs, with those born at 25 to 27 weeks GA displaying a blunted heart rate response to acute pain, and those born at 28 to 42 weeks GA displaying an increase in heart rate across phases. When the mean HR responses to acute pain were pooled by GA, they did not significantly differ in those born at 28-32, 32-34, 34-37, and 37-42 weeks GA in the first 7 postnatal days of life. However, these pooled mean HR responses were significantly different clinically, as the pooled mean HR responses would lead to different pain score categories on certain multimodal measures of pain for the preterm and term born infants.

Mean Heart Rate Change: Mean HR change was utilized in studies investigating infants born at 28 to 32 weeks and 37 to 42 weeks GA. Both groups had a significant increase in HR following the acutely painful procedure. Maximum HR in response to a heel stick was utilized in infants' born at 37 to 42 weeks GA. All studies found that maximum HR increased in response to the heel stick.

Heart Rate Variability: Total HRV, LF HRV, HF HRV, and the LF/HF ratio were examined in infants born at 28 to 32, 32 to 34, and 37 to 42 weeks GA. Although LF and HF HRV were found to increase, and the LF/HF ratio decreased in response to acute pain in one study investigating those born at 28 to 32 weeks GA, clear patterns of HRV in response to acute pain could not be deciphered in the later born infants (i.e., 32 to 34 and 37 to 42 weeks GA).

3.4. Age at Measurement: One to Two Postnatal Weeks

3.4.1. Extremely preterm

No studies investigated cardiovascular responses to acute pain in extremely preterm infants in the first or second postnatal week of life.

3.4.2. Very preterm

28 to 32 Weeks GA.

Mean HR Change: One lower quality study investigated the mean change in HR following a heel stick at one postnatal week (Goubet et al., 2001). The authors found that infants' HR increased by approximately 5 to 10 bpm during the most invasive event of the blood collection. One study investigated mean HR change following a heel stick procedure in infants who were less than 14 postnatal days old (Cong et al., 2012). During the procedure the authors found that mean HR increased significantly from baseline to heel stick procedure, with a mean HR change of 22.40 bpm, and standard deviation of 15.42.

Heart Rate Variability: One high quality study investigated several components of HRV in response to a heel stick at 14 postnatal days or less (Cong et al., 2012). The authors reported that LF and HF HRV increased in response to heel stick, while the LF/HF ratio decreased in response to heel stick. At the time of heel stick, mean LF HRV was reported at 69.84, with a

standard deviation of 102.08, mean HF HRV was reported at 24.04, with a standard deviation of 40.90, and the LF/HF ratio was reported at 23.98, with a standard deviation of 21.39.

3.4.3. Moderate to late preterm

32 to 35 Weeks GA

Mean Heart Rate: One relatively high quality study investigated mean HR in response to a heel stick at less than 10 postnatal days (Johnston et al., 2007). Mean heart rate was found to increase in response to the heel stick, and was approximately 159 bpm in response to the acute pain. A variety of covariates were included in the analysis of the cardiovascular measure, which included Apgar scores at 5 minutes, GA at birth, time since last painful procedure, number of painful procedures since admission, or received indomethacin in the past 12 hours (yes/no).

3.4.4. Full term

Mean Heart Rate: Two studies investigated mean HR response to heel stick (Craig et al., 1993) or venipuncture (Upadhyay et al., 2004) at less than 15 postnatal days old. Mean HR was found to significantly increase in response to venipuncture, and was reported at 163 bpm following the acutely painful procedure. In response to heel stick, mean HR was found to increase to 145.86 bpm with a standard deviation of 19.22 (Craig et al., 1993). Overall, the quality of the studies was high.

3.4.5. Summary

Mean Heart Rate: Data were available from studies investigating those born at 28 to 32, 32 to 35, and 37 to 42 weeks GA during the second postnatal week. Mean HR significantly increased in response to acutely painful procedures. The magnitude of heart rate responses was variable within and across GA groups.

Heart Rate Variability: One study found that infants born at 28 to 32 weeks GA were found to have increased LF and HF HRV, and a decreased LF/HF ratio in response to acute pain.

3.5. Age at Measurement: Three Postnatal Weeks

3.5.1. Extremely preterm

24 to 26 Weeks GA. One high quality study investigated the mean and maximum HR response following a heel stick at 21 postnatal days (Walden et al., 2001). The authors found that mean and maximum HR increased during the heel stick, and was reported as 174.90 bpm with a standard deviation of 9.86 bpm, and 175.91 bpm with a standard deviation of 10.35 bpm, respectively.

3.5.2. Very preterm

No studies investigated cardiovascular responses to acute pain in very preterm infants in the third postnatal week of life.

3.5.3. Moderate to late preterm

No studies investigated cardiovascular responses to acute pain in moderate to late preterm infants in the third postnatal week of life.

3.5.4. Full term

No studies investigated cardiovascular responses to acute pain in full term infants in the third postnatal week of life.

3.5.5. Summary

Mean Heart Rate: In extremely preterm infants, mean and maximum HR were found to increase in response to acute pain at 3 postnatal weeks old. The blunted heart rate response that was noted in the first seven postnatal days was not found, suggesting an increased response to acute pain developing in extremely preterm infants in the first three weeks of life.

3.6. Age at Measurement: One Postnatal Month

3.6.1. Extremely preterm

24 to 28 Weeks GA.

Mean Heart Rate: Two studies with varying quality levels investigated mean HR following a heel stick procedure at four postnatal weeks (Johnston & Stevens, 1996; Oberlander et al., 2002b). The authors found that there were significant increases in mean HR following the heel stick. The approximate mean HR response following the heel stick ranged from 170 to 190 bpm. A variety of covariates were included in one analysis (Johnston & Stevens, 1996), which comprised frequency of invasive procedures, severity of illness, ventilation status, and sex.

Heart Rate Variability: One study investigated LF and HF HRV and the LF/HF ratio during a heel lance procedure (Oberlander et al., 2002b). LF and HF HRV as well as the LF/HF ratio decreased during heel lance, and were approximately 5.0, 1.0, and 8.0 during the heel lance, respectively.

3.6.2. Very preterm

28 to 32 Weeks GA.

Mean Heart Rate: One high quality study investigated mean HR following a heel stick at three to five postnatal weeks (Lucas-Thompson et al., 2008). The authors found that mean HR was significantly higher during the heel stick than during recovery, and was reported at 175.94 bpm, with a standard deviation of 12.66 bpm during the heel stick. The number of prior heel sticks, duration of blood draws, sex, and baseline heart rate were included as covariates in the analysis.

3.6.3. Moderate to late preterm

No studies investigated cardiovascular responses to acute pain in moderate to late preterm infants in the first postnatal month of life.

3.6.4. Full term

No studies investigated cardiovascular responses to acute pain in full term infants in the first postnatal month of life.

3.6.5. Summary

Mean Heart Rate: Data from studies investigating infants at 24 to 28 and 28 to 32 weeks GA were available. Both studies found that mean HR increased in response to acute pain. Mean HR at one postnatal month was higher in response to acute pain, as compared to the first 7 postnatal days in those born 28 to 32 weeks GA.

Heart Rate Variability: At one postnatal month, one study found that LF and HF HRV and the LF/HF ratio decreased in response to acute pain.

3.7. Age at Measurement: Two Postnatal Months

3.7.1. Preterm Infants

No studies investigated cardiovascular responses to acute pain in extremely, very, moderate to late preterm infants in the second postnatal month of life.

3.7.2. Full term

Mean Heart Rate: One relatively lower quality study investigated mean HR responses to a heel stick procedure at two postnatal months (Oberlander et al., 2002). The authors found that mean HR increased post heel stick, and was approximately 190 bpm during the heel stick. The authors included a variety of covariates in their analysis (i.e., breast-fed (yes/no), SSRI exposure

(yes/no), age at time of acute pain, maternal analgesia (yes/no), dose of SSRI at time of delivery, dose of clonazepam at time of delivery.

Heart Rate Variability: The same study investigated mean HRV during the aforementioned acute pain procedure (Oberlander et al., 2002). The authors found that during the heel stick procedure, LF HRV and the LF/HF ratio decreased, however, there were no significant differences in HF HRV. HF and LF HRV, as well as the LF/HF ratio were approximately 4.0, 28.0, and 8.0 during the heel stick procedure, respectively. The abovementioned covariates were used in the analysis.

3.7.5. Summary

Mean Heart Rate: One study investigated those born at 37 to 42 weeks GA. Mean HR was found to increase in response to acute pain.

Heart Rate Variability: Although LF HRV and the LF/HF ratio were found to decrease in response to pain, HF HRV was not significantly different from baseline to heel stick.

3.8. Age at Measurement: Three Postnatal Months

3.8.1 Preterm Infants.

No studies investigated cardiovascular responses to acute medical procedure pain in extremely preterm, very preterm, moderate to late preterm infants in the third postnatal month of life.

3.8.2. Full term

Mean Heart Rate: One relatively lower quality study investigated mean HR response following a heel stick at three postnatal months (Lindh et al., 2003). Mean HR increased post heel stick and was approximately 169 bpm during this time.

Heart Rate Variability: The same study investigated mean HRV during the aforementioned heel stick procedure (Lindh et al., 2003). In the study, the authors found that total HRV and the LF HRV increased during the heel stick, however there were no significant differences in HF HRV compared to baseline. When extrapolating the values, total HRV, HF and LF HRV were approximately 4.10, 3.20, and 4.0 during the heel stick procedure, respectively.

3.8.5. Summary

Mean Heart Rate: Data from one lower quality study investigating those born at 37 to 42 weeks GA were available. Mean HR was found to increase in response to acute pain.

Heart Rate Variability: Although total and LF HRV were found to increase in response to pain, HF HRV was not significantly different from baseline to heel stick.

3.9. Age at Measurement: Four Postnatal Months

3.9.1. Extremely preterm

24 to 28 Weeks GA

Mean Heart Rate: One relatively higher quality study investigated mean HR response following immunizations at four postnatal months (Grunau et al., 2010). The authors found that mean HR changed significantly across events, with significant increases from the end of baseline to first injection, and first injection to third injection. The approximate mean HR during the immunizations was 185 bpm. Corrected chronological age was included as a covariate in the analysis.

3.9.2. Very preterm

28 to 32 Weeks GA

Mean Heart Rate: The same study investigated mean HR response following immunizations at four postnatal months in infants born at 29 to 32 weeks GA (Grunau et al., 2010). The authors found that mean HR changed significantly across events, with significant increases from the end of baseline to first injection, and first injection to third injection. The approximate mean HR during the immunizations was 188 bpm. The abovementioned covariate was included in the analysis.

3.9.3. Moderate to late preterm

No studies investigated cardiovascular responses to acute pain in moderate to late preterm infants in the fourth postnatal month of life.

3.9.4. Full term

Mean Heart Rate: One study investigated mean HR response following immunizations at four postnatal months in infants born at 38 to 41 weeks GA (Grunau et al., 2010). The authors found that mean HR changed significantly across events, with significant increases from the end of baseline to first injection, and first injection to third injection. The approximate mean HR during the immunizations was 182 bpm. The abovementioned covariate was included in the analysis.

3.9.5. Summary

Only one cross-sectional, relatively higher quality study (Grunau et al., 2010) investigated the effect of GA on mean HR response following immunizations. The authors found that there was no effect of GA group (i.e., 24-28, 29-32, and 38-41 weeks GA) on mean HR response.

4. Discussion

The purpose of the current review and meta-analysis was to better understand the development of cardiovascular responses to acute pain over the first year of life based on GA. We also aimed to contrast the available literature to see how acute pain related cardiovascular responses differ in those born prematurely compared to those born at term. By way of overview, when measuring heart rate, maximum heart rate or heart rate change in the first 7 days of life, the range within each age group on these measures became larger as the infant's GA increased. Measures of heart rate variability in the first 7 days of life (using LF HRV or HF HRV) seemed to show less variability within age categories as the child's GA increased, while there was a trend towards less variability within an age category in the LF/HF ratio as the infants aged. Data from other postnatal age groups (i.e. 2nd week, 3rd week, 1 month, 2 month and 3 months) were very sparse with patterns generally impossible to discern due to the total absence or presence of 1 study.

The following paragraphs will discuss key findings and patterns in the results of the systematic review and meta-analysis with specific attention to GA at birth, age at measurement and type of cardiac measurement in response to acutely painful procedures. Comparisons will then be drawn across GA groups depending on postnatal age at measurement. Finally, limitations and strengths of the review, and key areas for future research based on the findings will be highlighted.

4.1. Extremely preterm

Studies included infants born extremely premature (i.e.,< 28 weeks GA) who were measured at less than seven postnatal days, three postnatal weeks, as well as one and four postnatal months. Those born at less than 28 weeks GA displayed a blunted heart rate response

to acute pain in the first week of life. By the third and fourth postnatal weeks of life, mean and maximum heart rate were found to significantly increase during acutely painful procedures, as compared to baseline heart rate. At one postnatal month of life, LF and HF HRV and the LF/HF ratio decreased in response to acute pain. Based on one cross-sectional study, this pattern of increase was also seen at four postnatal months, where mean HR significantly increased from baseline to acutely painful procedure, and was qualitatively higher than during the first postnatal month. This synthesis suggests that mean HR responses to acute pain may be stable in extremely preterm infants after the first postnatal week of life.

A blunted pain response in the first week of life based on GA a birth has been noted in both physiological and behavioural parameters (Craig et al., 1993; Johsnton, Stevens, Yang, & Horton, 1995; Stevens, Johnston, & Horton, 1993). It is possible that the health status of the child at birth may affect the infants' ability to react to invasive procedures during the first week of life (Craig et al., 1993). Indeed, infants with the lowest GA have the most health problems (e.g., lower birth weights, more oxygen received at delivery, greater use of ventilators at delivery and during observation, and greater use of gavage feeding) (Craig et al., 1993), are exposed to more invasive procedures (e.g., blood sampling, steroid exposure, suctioning, and routines such as weighing and clustered nursing) (Glover, Miles, Matta, Modi, & Stevenson, 2005; Holsti, Weinberg, Whitfield, & Grunau, 2007), and are the most developmentally immature (Grunau et al., 2010).

The increase in cardiac responding from baseline that was seen consistently in the first to the fourth postnatal month of life suggests that extremely low GA infants begin to demonstrate increased physiological responses to acute pain as the cardiovascular system matures (Sugihara et al., 1996). Indeed, past research has shown that infants born at 27 weeks GA or less do not

have complex or distinctly nonlinear heart rhythms that are consistent with healthy adults (Sugihara et al., 1996). Additionally, parasympathetic and sympathetic functions have been found to evolve between 27 and 35 weeks gestation (Allan & Sobel, 1992; Chatow, Davidson, Reichman, & Akselrod, 1995; Sugihara et al., 1996). Although the relative quality of studies was good (76 to 86%), it is important to interpret this qualitative synthesis with caution, given the relatively small group of studies ($N = 3$) that it integrates and the lack of covariates in two of the three studies.

4.2. Very preterm

Studies included very preterm (i.e., 28 to < 32 weeks GA) infants measured at less than seven postnatal days, one to two postnatal weeks, and one and four postnatal months. In the first week of life, mean HR was found to significantly increase immediately following an acutely painful procedure. From one to two postnatal weeks, mean HR was found to significantly increase in response to acute pain. Significant increases in mean HR were also described at three to five postnatal weeks, which were qualitatively higher than at one to two postnatal weeks of life. At four postnatal months, infants were found to have significant increases in mean HR in response to acute pain. The magnitude of the mean HR response was found to qualitatively increase from one to four postnatal months of life. As mentioned above with extremely preterm infants, this qualitative increase in mean HR in very preterm infants across the first four postnatal months are likely linked to a developmental, relative increase in the parasympathetic contribution to HR control (Allan & Sobel, 1992; Chatow et al., 1995; Sugihara et al., 1996).

Although HRV components were only investigated in one study of very preterm infants in the second postnatal week of life, LF and HF HRV were found to increase, while the LF/HF ratio decreased in response to acute pain. This pattern is in line with previous research that found

that LF and HF HRV increase during painful procedures (Grunau et al., 2001; Lindh et al., 1999; Padhye, Williams, Khattak, & Lasky, 2009). Caution should be taken when interpreting these HR and HRV results, as it is based on four studies (quality scores range from 62 to 85%), and a single study (quality score: 85%), respectively. Additionally, only one study included covariates in their analysis.

4.3. Moderate to late preterm

Cardiovascular indices of acute pain were only examined during the first and second postnatal weeks of life in infants born at 32 to less than 37 weeks GA. During the first postnatal week of life, mean HR was found to increase in response to acute pain; however, the magnitude of responses was variable. Mean HR was found to be stable across the second week of life, and increased in response to acute pain. The inconsistencies in mean HR may be due to differences in the acute pain procedure (i.e. heel stick versus venipuncture), the variability in quality of studies (40 to 85%), and the lack of covariates included in the analyses of more than half of the studies (3/5).

Additionally, when total HRV in response to acute pain was examined in the first week of life in those born at 32 to 34 weeks GA, it did not significantly differ from baseline HRV. Given that non-linearity in heartbeats, which is necessary to measure HRV, is less apparent before 35 weeks GA (Sugihara et al., 1996), it is possible that the variance in beat-to-beat rhythms is not robust enough to characterize the acutely painful procedure. Moreover, the conclusions are based on one study, and although the quality was adequate (75%), there were no covariates included in the analysis.

4.4. Full term

Full term infants' response to acute pain, as indexed by cardiovascular responses, was investigated in the first and second postnatal weeks of life, and during the second, third, and fourth postnatal month. During the first postnatal week of life, full term infants displayed an increase in mean and maximum HR in response to acute pain; however, the magnitude of responses was variable. At two postnatal weeks, full term infants displayed an increase in mean HR following an acutely painful procedure; although the studies displayed variable results that qualitatively differed based on mean HR in bpm. At two, three and four postnatal months, full term infants displayed an increase in mean HR in response to acute pain that peaked during postnatal month two. This relative increase in mean HR over the first four postnatal months may reflect a developmental, relative increase in the parasympathetic contribution to HR control (Chatow et al., 1995). Conversely, the variability in mean HR response across the first four months of life may be due to extraneous factors not being accounted for in the majority of analyses discussed. For example, respiration has been found to affect modulation of instantaneous HR (Chatow et al., 1995). However, this was not included in any of the analyses as a covariate. Furthermore, there was only one study per age group at two, three and four postnatal months, the study authors included differing or no covariates in their analyses, and the study quality ranged from 55 to 76%.

During the first postnatal week of life, total HRV was found to be inconsistent, and increased and decreased in response to acute pain. In regards to HF HRV, it was also unreliable, with studies finding that that it decreased or did not significantly change in response to acute pain. LF HRV was also variable, with one study finding it decreased in response to acute pain, while the other study found it increased in response to acute pain. The LF/HF ratio was the only

consistent measure of HRV, and it was found to decrease in response to acute pain across studies.

At two postnatal months, LF HRV and the LF/HF ratio decreased in response to acute pain. There were no significant differences in HF HRV in response to acute pain at two postnatal months of age. At three months of age in response to acute pain, total and LF HRV increased, while no differences were found in HF HRV when compared to baseline levels. The synthesis suggests that there is stability in the LF/HF ratio from one week to two months of age. However, due to this measure being a product of the LF and HF HRV values, all measures of HRV are inconsistent across the time points investigated (i.e., postnatal week one to month four).

The inconsistency within the HRV domains may be explained by the linear statistics utilized by authors (Sugihara et al., 1996). Specifically, past research has found that nonlinear analysis is superior in discriminating differences in infant heart rhythms when compared to linear statistics, such as the mean or variance between heartbeats (Sugihara et al., 1996). As well, only one study out of eight included covariates in their analysis, and the quality of the studies varied qualitatively (55 to 80%).

4.5. Comparing preterm and term born infants

Due to a lack of research across gestational and postnatal ages, a clear comparison of the development of cardiovascular responses to acute pain over the first year of life was not possible. However, short summaries of the qualitative and quantitative differences in preterm and full term infants are described for less than seven postnatal days and four postnatal months.

During the first postnatal week of life, those born extremely preterm (25 to 27 weeks GA) were found to have a blunted cardiovascular response to acute pain, as compared to infants born very preterm (28 to 30 weeks GA), moderate to late preterm (31 to 33 and 34 to 36 weeks GA),

and full term (37 to 47 weeks GA) (Craig et al., 1993). Qualitatively, there was greater variability in mean HR responses to acute pain as GA increased, which has been substantiated in previous behavioural pain research in term born infants (Pillai Riddell et al., 2013). Additionally, mean HR in response to acute pain was higher in full term compared to moderate to late preterm infants (Singh et al., 2000). Finally, in our own quantitative analysis, pooled mean HR responses to acute pain were not statistically different in those born at varying levels of prematurity or term. However, it is important to note that these pooled means were clinically significant, as the pooled means would lead to infants at differing GA groups being assigned to separate pain categories on several assessment tools (e.g., PIPP-R). These inconclusive results are found in the general infant pain literature, which has established that preterm infants respond in a manner similar to full term neonates (Stevens, Johnston, & Horton, 1993), as well as responding less robustly than full term neonates (Johnston et al., 1995).

During postnatal month four, regardless of GA at birth (i.e., extremely preterm, very preterm or full term), infants displayed an increase in mean HR in response to acute pain. Moreover, the magnitude of mean HR in response to acute pain did not significantly differ based on GA at birth. The results suggest that as the cardiovascular system develops over the first four postnatal months of life, mean HR responses to acute pain become similar in those born at differing levels of prematurity and at term. However, it is possible that by using an overall mean HR to represent infant pain responses, it may have led to misrepresentation of stable subgroups within these groups of infants (Pillai Riddell et al., 2013).

4.6. Limitations of this review

It is possible that we have omitted relevant studies despite our detailed search strategy, and we specifically excluded non-English language studies. Additionally, group-specific data

(i.e., age at measurement and GA) were separated based on available data and natural groupings, which on occasion led to overlap in GA groups. Furthermore, pooling the means and standard deviations of cardiovascular measures based on GA group would have allowed us to investigate differences in how the cardiovascular system develops in preterm compared to term born infants. However, there are too few studies for this approach to be done, and it could only be completed for one-week-old infants.

Moreover, differences in the methodology for both acquiring and analyzing HR and HRV data led to difficulties in making comparisons of cardiovascular measures between GA groups and postnatal ages (Padhye et al., 2009). Specifically, there was notable variability in how mean HR, HR change, and HRV were calculated over post-acute pain (See Table 11), which may have led to increased variability in the cardiovascular measures. Additionally, this variability in the timing of events is a limitation for our pooled analyses, as the pooled responses may not being measuring the same event. With regards to analyzing HRV, studies differed on spectrum calculation methods and models of data analysis. Although terminology such as LF and HF bands is common in the field, studies differ on frequency limits of the bands. Other studies utilized linear statistical approaches of comparing means and variance, which has been reported as less sensitive in classifying HRV in infants (Chatow et al., 1995).

Furthermore, it was difficult to draw conclusions across development and GA groups for cardiovascular responses to acute medical procedure pain, as the majority of studies did not include covariates in their analyses that could impact an infants' cardiovascular response to acute pain. It is important to keep in mind that the variability in mean HR and HRV components may be due to this lack of control within the studies. Finally, the majority of the studies reviewed in

the study were very small sample sizes, all of which were too small to begin to examine variability within a GA category at any postnatal age more in-depth.

4.7. Review Contributions to the Literature

To our knowledge, this is the first systematic review and meta-analysis investigating the development of cardiovascular indices of acute pain responding across the first year of life. A particular contribution of the methodology pursued was that GA, age at measurement (postnatal age), and type of cardiac response was analyzed separately. This is the first systematic review and meta-analysis that provides mean and variance for all cardiovascular indices utilized in observational and experimental studies of acute pain in infancy. In doing so, the results have begun the process of better describing the development of cardiovascular responses to acute medical procedure pain. The large gaps elucidated in this review can provide a framework for future research in the field. In particular, understanding the development of infant pain responding outside of the first month of life, with attention to GA at birth still remains largely unknown. Finally, a comprehensive list of covariates utilized in each study has been given, which provides researchers with guidelines for future research studies.

4.8. Implications for research and clinical practice

In the current review, 41 studies were examined in order to better understand how those born prematurely and at term respond to acute pain, as indexed by cardiovascular indices. Research across the first four months of life suggest that most infants have an increase in mean HR in response to acute pain across gestational and postnatal ages but that the variability within a GA category (i.e., extremely preterm, very preterm, moderate to late preterm, full term) increases as the GA increases. The presence of variability in heart rate or heart rate change in older preterm infants and full term infants presents an important clinical challenge to gold

standard measures such as the PIPP-R, N-PASS, COMFORT and Bernese Pain Scale. These scales allocate points to preterm infants' pain score in order to approximate the normal response of a full-term infant. However, the synthesis of our review suggests that the variability in heart rate responding increases with GA at birth.

Research in the area of HRV has been inconsistent, and no conclusions can be drawn. Furthermore, there is a great amount of variability in the magnitude of HR and HRV responses to acute pain within and across GA groups and postnatal age at measurement.

The results were mixed regarding differences in cardiovascular responses to acute pain within the first week of life based on GA group. Pooled means in our meta-analysis did not suggest statistically significant differences between mean HR response to acute pain in those born at 28 to 32, 32 to 34, 34 to 37, and 37 to 42 weeks GA in the first week of life. However, when investigating the individual variability within GA category across the first week of life, there are clinically significant increases in the variability of mean HR responses as GA at birth increases. Additionally, one study found that those born extremely preterm (25 to 27 weeks GA) have a blunted mean HR response to acute pain in the first week of life. Moreover, in another study during the first postnatal week of life, very preterm and moderate to late preterm infants were found to have an increase in mean HR that was less robust than those born at term. At four postnatal months, although mean HR increased in response to acute pain across GA groups (24-28, 29-32, and 38-41 weeks GA), GA had no effect on mean HR response.

This information is important for health care providers and researchers, as it suggests that cardiovascular indices can vary significantly across gestational and postnatal age groups, and should be used in conjunction with behavioural measures to ensure infant pain is being assessed and managed properly. It is also important to emphasize that although a blunted cardiovascular

response to acute pain in extremely preterm infants was noted, it does not mean that these infants are not experiencing pain. Health factors that are specific to those born extremely preterm are additional sources of variation in response to acute pain, and we emphasize the need for future research that addresses this group of infants systematically (Grunau et al., 2010).

A lack of control within the studies investigated has been highlighted, with only 13 out of 41 studies including covariates in their analysis of cardiovascular responses to acute pain. Moreover, the covariates utilized in the studies are divergent, which may have increased the amount of variability noted in the cardiovascular responses to acute pain. Future research in the area of infant pain should address this lack of control by identifying and controlling for factors that may affect an infants' cardiovascular response to acute pain in their own research. Examining the studies that did use covariates, key covariates that should seriously be considered for inclusion in all cardiac response to pain studies (depending on design) are: gestational age, age at measurement (i.e., postnatal age, corrected chronological age), birth weight, time since last feeding, ventilation status, baseline (i.e. pre-handling cardiac responding), length of painful procedure, number of painful procedures (e.g. how many draw attempts), illness severity, sex, and respiration rate.

Additionally, variability in the analysis of HRV measures has been noted above, and it is important to emphasize that measures specifically characterizing the nonlinearity of heart-rate time series, and not just their means and variance, may provide more direct and sensitive methods for assessing the physiological state of infants (Chatow et al., 1995).

Overall, our results highlight important gaps where additional research is needed. There is a lack of research investigating cardiovascular responses to acute pain in specific gestational and postnatal age groups of infants across the first years of life. This is noteworthy, as studies are

not attempting to accommodate the inherent variability of cardiovascular pain responses and the causes for the variability (Pillai Riddell et al., 2013).

References

Abad, F., Diaz-Gomez, N. M., Domenech, E., Gonzalez, D., Robayna, M., & Feria, M. (2001). Oral sucrose compares favourably with lidocaine-prilocaine cream for pain relief during venepuncture in neonates. *Acta Paediatrica, 90,* 169-165.

Altun-Köroğlu, O., Ozek, E., Bilgen, H., & Cebeci, D. (2009).Hindmilk for procedural pain in term neonates. *The Turkish Journal of Pediatrics*, *52*, 623-629.

Allan, W. C., & Sobel, D. B. (1992).The sans of time. *JAMA*, *268*, 984-984.

Ambuel, B., Hamlett, K. W., Marx, C. M., & Blumer, J. L. (1992). Assessing distress in pediatric intensive care environments: the COMFORT scale. *Journal of Pediatric Psychology*, *17*, 95-109.

Bergqvist, L. L., Katz-Salamon, M., Hertegård, S., Anand, K. J. S., & Lagercrantz, H. (2009). Mode of delivery modulates physiological and behavioral responses to neonatal pain. *Journal of Perinatology*, *29*, 44-50.

Bilgen, H., Özek, E., Cebeci, D., & Örs, R. (2001).Comparison of sucrose, expressed breast milk, and breast-feeding on the neonatal response to heel prick. *The Journal of Pain*, *2*, 301-305.

Bucher, H. U., Baumgartner, R., Bucher, N., Seiler, M., & Fauchere, J. C. (2000). Artificial sweetener reduces nociceptive reaction in term newborn infants. *Early Human Development*, *59*, 51-60.

Booth, A. PROSPERO: International register of systematic reviews. Available at: http://www.crd.york.ac.uk/PROSPERO; 2013.

Bouza, H. (2009). The impact of pain in the immature brain. *Journal of Maternal-Fetal and Neonatal Medicine*, *22*, 722-732.

Brummelte, S., Grunau, R. E., Chau, V., Poskitt, K. J., Brant, R., Vinall, J., ... & Miller, S. P. (2012). Procedural pain and brain development in premature newborns. *Annals of Neurology*, *71*, 385-396.

Cabal, L. A., Siassi, B., & Hodgman, J. E. (1992).Neonatal clinical cardiopulmonary monitoring. In A. A. Fanaroff & R. J. Martin (Eds.), *Neonatal-Perinatal Medicine* (pp. 437-455). Mosby, St. Louis: Mosby Year Book.

Campos, R. G. (1994). Rocking and pacifiers: Two comforting interventions for heelstick pain. *Research in Nursing, 17*, 321-331.

Chatow, U., Davidson, S., Reichman, B. L., & Akselrod, S. (1995). Deveopment and maturation of the autonomic nervous system in premature and full-term infants using spectral analysis of heart rate fluctuations. *Pediatric Research, 37,* 294-302.

Cignacco, E., Mueller, R., Hamers, J. P., & Gessler, P. (2004). Pain assessment in the neonate using the Bernese Pain Scale for Neonates. *Early Human Development*, *78*, 125-131.

Cong, X., Ludington-Hoe, S. M., McCain, G., & Fu, P. (2009). Kangaroo care modifies preterm infant heart rate variability in response to heel stick pain: Pilot study. *Early Human Development, 85*, 561-567.

Cong, X., Cusson, R. M., Walsh, S., Hussain, N., Ludington-Hoe, S. M., & Zhang, D. (2012).Effects of skin-to-skin contact on autonomic pain responses in preterm infants.*The Journal of Pain, 17*, 636-645.

Craig, K., Whitfield, M. F., Grunau, R. V. E., Linton, J., & Hadjistavropoulos, H. D. (1993). Pain in the preterm neonate: Behavioural and physiological indices. *Pain, 52*, 287-299.

Crombie, I. K., & McQuay, H. J. (1998). The systematic review: a good guide rather than a guarantee. *Pain*, *76*, 1-2.

de Jesus, J. A., Tristao, R. M., Storm, H., da Rocha, A. F. & Campos, D. J. (2011). Heart rate, oxygen, saturation, and skin conductance: A comparison study of acute pain in Brazilian newborns. Conference Proceedings from *the IEEE Engineering in Medicine and Biology Society.* Paper presented at the 33rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Boston, Massachusetts (pp. 1875-1879). Boston, MA: IEEE EMBS.

Denk, F., McMahon, S. B., & Tracey, I. (2014). Pain vulnerability: a neurobiological perspective. *Nature neuroscience*, *17*(2), 192-200.

de Oliveira, M. V. M., de Jesus, J. A. L., & Tristao, R. M. (2012). Psychophysical parameters of a multidimensional pain scale in newborns.*Physiological Measurement, 33*, 39-49.

Downs, S. H., & Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *Journal of epidemiology and community health*, *52*, 377-384.

Dykes, F. D., Ahmann, P. A., Baldzer, K., Carrigan, T. A., Kitney, R., & Giddens, D. P. (1986). Breath amplitude modulation of HR variability in normal full term neonates. *Pediatric Research, 20,* 301-308.

Faye, P. M., De Jonckheere, J., Logier, R., Kuissi, E., Jeanne, M., Rakza, T., & Storme, L. (2010). Newborn infant pain assessment using heart rate variability analysis. *The Clinical journal of pain*, *26*, 777-782.

Gibbins, S., Stevens, B. J., Yamada, J., Dionne, K., Campbell-Yeo, M., Lee, G., ... & Taddio, A. (2014). Validation of the Premature Infant Pain Profile-Revised (PIPP-R). *Early human development*, *90*, 189-193.

Glover, V., Miles, R., Matta, S., Modi, N., & Stevenson, J. (2005). Glucocorticoid exposure in preterm babies predicts saliva cortisol response to immunization at 4 months. *Pediatric Research, 58,*1233-1237.

Gormally, S., Barr, R. G., Wertheim, L., Alkawaf, R., Calinoiu, N., & Young, S. N. (2001). Contact and nutrient caregiving effects on newborn infant pain responses. *Developmental Medicine and Child Neurology, 43*, 28-38.

Goubet, N., Clifton, R. K., & Shah, B. (2001). Learning about pain in preterm newborns. *Journal of Developmental and Behavioral Pediatrics, 22*, 418-424.

Gray, L., Watt, L., & Blass, E. M. (2000). Skin-to-skin contact is analgesic in healthy newborns. *Pediatrics, 105*, e14.

Greenberg, C. S. (2002). A sugar-coated pacifier reduces procedural pain in newborns. *Pediatric Nursing, 22*, 271-277.

Grunau, R. (2013). Long-term effects of pain in children. In P. McGrath, B. Stevens, S. Walker, & W. Zempsky (Eds.), *Oxford Textbook of Paediatric Pain* (pp. , 30-38). Oxford, UK: Oxford University Press.

Grunau, R. E., Holsti, L., & Peters, J. W. (2006). Long-term consequences of pain in human neonates.In *Seminars in Fetal and Neonatal Medicine* (Vol. 11, No. 4, pp. 268-275). WB Saunders.

Grunau, R. E., Oberlander, T. F., Whitfield, M. F., Fitzgerald C., & Lee S. K. (2001). Demographic and therapeutic determinants of pain reactivity in very low birth weight neonates at 32 weeks postconceptional age. *Pediatrics*, *107*, 105-112.

Grunau, R. E., Tu, M. T., Whitfield, M. F., Oberlander, T. F., Weinberg, J., Yu, W., … Scheifele, D. (2010). Cortisol, behavior, and heart rate reactivity to immunization pain at

4 months corrected age in infants born very preterm. *Clinical Journal of Pain, 26*, 698-704.

Grunau, R. V. E., Whitfield, M. F., & Petrie, J. H. (1994) Pain sensitivity and temperament in extremely-low-birth-weight premature toddlers and preterm and full-term controls. *Pain,58*, 341-346.

Grunau, R. E., Whitfield, M. F., Petrie-Thomas, J., Synnes, A. R., Cepeda, I. L., Keidar, A., ... & Johannesen, D. (2009).Neonatal pain, parenting stress and interaction, in relation to cognitive and motor development at 8 and 18months in preterm infants. *Pain*, *143*, 138-146.

Haouari, N., Wood, C., Griffiths, G., & Levene, M. (1995). The analgesic effect of sucrose in full term infants: A randomized controlled trial. *British Medical Journal, 310*, 1498-1500.

Hazinski, M. F. (1990).Shock in the pediatric patient. *Critical care nursing clinics of North America*, *2*, 309-324.

Holsti, L., Weinberg, J., Whitfield, M. F., & Grunau, R. E. (2007). Relationships between adrenocorticotropic hormone and cortisol are altered during clustered nursing care in preterm infants born at extremely low gestational age. *Early Human Development, 83,* 341-348.

Hummel, P., Lawlor-Klean, P., & Weiss, M. G. (2010). Validity and reliability of the N-PASS assessment tool with acute pain. *Journal of Perinatology*, *30*, 474-478.

Jatana, S. K., Dalal, S. S., & Wilson, C. G. (2003). Analgesic effect of oral glucose in neonates.*Medical Journal Armed Forces India, 59*, 100-104.

Johnston, C. C., Filion, F., & Nuyt, M. A. (2007).Recorded maternal voice for preterm neonates undergoing heel lance.*Advances in Neonatal Care, 7*, 258-266.

Johnston, C. C., & Stevens, B. J. (1996). Experience in a neonatal intensive care unit affects pain response. *Pediatrics, 98*, 925-930.

Johnston, C. C., Stevens, B., Yang, F., & Horton, L. (1995). Developmental changes in response to heelstick in preterm infants: A prospective cohort study. *Developmental Medicine and Child Neurology, 38*, 438-445.

Kostandy, R., Anderson, G. C., & Good, M. (2013). Skin-to-skin contact diminishes pain from Hepatitis B Vaccine injection in healthy full-term neonates. *Neonatal Network, 32*, 274-280.

Kristjánsdóttir, Ó., Unruh, A. M., McAlpine, L., & McGrath, P. J. (2012). A systematic review of cross-cultural comparison studies of child, parent, and health professional outcomes associated with pediatric medical procedures.*The Journal of Pain*, *13*(3), 207-219.

Leite, A. M., Linhares, M. B. M., Lander, J., Castral, T. C., dos Santos, C. B., & Scochi, C. G. S. (2009). Effects of breastfeeding on pain relief in full-term newborns.*Clinical Journal of Pain, 25*, 827-832.

Lindh, V., Wiklund, U., Blomquist, H. K., & Hakansson, S. (2003).EMLA® cream and oral glucose for immunization pain in 3-month-old-infants.*Pain, 104,* 381-388.

Lindh, V., Wiklund, U., & Hakansson, S. (2000).Assessment of the effect of EMLA® during venipuncture in the newborn by analysis of heart rate variability.*Pain, 86,* 247-254.

Lindh, V., Wiklund, U., & Hakansson, S. (1999). Heel lancing in term new-born infants: an evaluation of pain by frequency domain analysis of heart rate variability. *Pain*, *80*, 143-148.

Lucas-Thompson, R., Townsend, E. L., Gunnar, M. R., Georgieff, M. K, Guiang, S. F., Ciffuentes, R. F., … Davis, E. P. (2008).*Infant Behavior and Development, 31*, 614-623.

Maxwell, L. G., Malavolta, C. P., & Fraga, M. V. (2013). Assessment of pain in the neonate. *Clinics in perinatology*, *40*, 457-469.

Mitchell, A., Brooks, S., & Roane, D. (2000).The premature infant and painful procedures. *Pain Management Nursing*, *1*, 58-65.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medicine*, *151*, 264-269.

Morison, S. J., Grunau, R. E., Oberlander, T. F., & Whitfield, M. F. (2001). Relations between behavioral and cardiac autonomic reactivity to acute pain in preterm neonates. *The Clinical journal of pain*, *17*, 350.

Oberlander, T. F., Grunau, R. E., Fitzgerald, C., Ellwood, A. L., Misri, S., Rurak, D., & Riggs, K. W. (2002). Prolonged prenatal psychotropic medication exposure alters neonatal acute pain response. *Pediatric Research, 51,* 443-453.

Oberlander, T. F., Grunau, R. E., Fitzgerald, C., Papsdorf, M., Rurak, D., & Riggs, W. (2005).Pain reactivity in 2-month-old infants after prenatal and postnatal selective serotonin reuptake inhibitor medication exposure.*Pediatrics, 115,* 411-425.

Oberlander, T. F., Grunau, R. E., Fitzgerald, C., & Whitfield, M. F. (2002b). Does parenchymal brain injury affect biobehavioral pain responses in very low birth weight infants at 32 weeks' postconceptional age? *Pediatrics, 110,* 570-576.

Ors, R., Ozek, E., Baysoy, G., Cebeci, D., Bilgen, H., Turkuner, M., & Basaran, M. (1999).Comparison of sucrose and human milk on pain response in newborns.*European Journal of Pediatrics, 158*, 63-66.

Owens, M. E., & Todt, E. H. (1984). Pain in infancy: Neonatal reaction to a heel lance. *Pain, 20*, 77-86.

Padhye, N. S., Williams, A. L., Khattak, A. Z., & Lasky, R. E. (2009). Heart rate variability in response to pain stimulus in VLBW infants followed longitudinally during NICU stay. *Developmental Psychobiology, 51,* 638-649.

Pillai Riddell, R., Flora, D. B., Stevens, S. A., Stevens, B., Cohen, L. L., Greenberg, S., & Garfield, H. (2013).Variability in infant acute pain responding meaningfully obscured by averaging pain responses. *PAIN*, *154*, 714-721.

Price, D. A., Close, G. C., & Fielding, B. A. (1983).Age of appearance of circadian rhythm in salivary cortisol values in infancy. *Archives of Disease in Childhood*, *58*, 454-456.Sajedi, F., Kashaninia, Z., Rahgozar, M., & Radrazm, L. (2006).The efficacy of oral glucose for relieving pain following intramuscular injection in term neonates.*Acta Medica Iranica, 44*, 316-322.

Ranger, M., Chau, C. M., Garg, A., Woodward, T. S., Beg, M. F., Bjornson, B., ... & Grunau, R. E. (2013). Neonatal pain-related stress predicts cortical thickness at age 7 years in children born very preterm. *PloS one*, *8*, e76702.

Rodkey, E. N., & Riddell, R. P. (2013). The infancy of infant pain research: the experimental origins of infant pain denial. *The Journal of Pain*, *14*, 338-350.

Rouzan, I. A. (2001).An analysis of research and clinical practice in neonatal pain management. *Journal of the American Academy of Nurse Practitioners*,*13*, 57-60.

Sanderson, S., Tatt, I. D., & Higgins, J. P. (2007). Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. *International journal of epidemiology*, *36*, 666-676.

Schwaller, F., & Fitzgerald, M. (2014). The consequences of pain in early life: injury‐induced plasticity in developing pain pathways. *European Journal of Neuroscience*, *39*, 344-352.

Shibata, M., Kawai, M., Matsukura, T., Heike, T., Okanoya, K., & Myowa-Yamakoshi, M. (2013). Salivary biomarkers are not suitable for pain assessment in newborns. *Early Human Development, 89*, 503-506.

Singh, H., Singh, D., & Soni, R. K. (2000). Comparison of pain response to venipuncture between term and preterm neonates. *Indian Pediatrics*, *37*, 179-181.

Slater, R., Cantarella, A., Franck, L., Meek, J., & Fitzgerald, M. (2008). How well do clinical pain assessment tools reflect pain in infants? *PLoS medicine*, *5*, e129.

Slater, R., Cornelissen, L., Fabrizi, L., Patten, D., Yoxen, J., Worley, A. ... & Fitzgerald, M. (2010). Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. *The Lancet*, *376*, 1225-1232.

Stevens, B. J., Gibbins, S., Yamada, J., Dionne, K., Lee, G., Johnston, C., & Taddio, A. (2014). The premature infant pain profile-revised (PIPP-R): initial validation and feasibility. *The Clinical Journal of Pain*, *30*, 238-243.

Stevens, B. J. & Johnston, C. C. (1994).Physiological responses of premature infants to a painful stimulus.*Nursing Research, 43,* 226-231.

Stevens, B. J., Johnston, C. C., & Grunau, R. V. (1995). Issues of assessment of pain and discomfort in neonates. *Journal of Obstetric, Gynecologic, & Neonatal Nursing*, *24*, 849-855.

Stevens, B. J., Johnson, C.C., & Horton L. (1993). Multidimensional pain assessment in premature neonates: A pilot study. *Journal of Obstetrics, Gynecology and Neonatal Nursing*, *22*, 531-541.

Stevens, B., Johnston, C., Petryshen, P., & Taddio, A. (1996). Premature Infant Pain Profile: development and initial validation. *The Clinical Journal of Pain*, *12*, 13-22.

Stevens, B. J., Pillai Riddell, R. R., Oberlander, T. E., & Gibbins, S. (2007). Assessment of pain in neonates and infants. *Pain in neonates and infants*, *5*, 67-90.

Sugihara, G., Allan, W., Sobel, D., & Allan, K. D. (1996).Nonlinear control of heart rate variability in human infants. *Proceedings of the National Academy of Sciences*, *93*, 2608-2613.

Taddio, A., Katz, J., Ilersich, A. L., & Koren, G. (1997). Effect of neonatal circumcision on pain response during subsequent routine vaccination. *The Lancet*, *349*, 599-603.

Taksande, A. M., Vilhekar, K. Y., Jain, M., & Chitre, D. (2005).Pain response of neonates to venipuncture. *The Indian Journal of Pediatrics*, *72*, 751-753.

Upadhyay, A., Aggarwal, R., Narayan, S., Joshi, M., Paul, V. K., & Deorari, A. K. (2004). Analgesic effect of expressed breast milk in procedural pain in term neonates: a randomized, placebo‐controlled, double‐blind trial. *Acta Paediatrica*, *93*, 518-522.

Uyan, Z. S., Özek, E., Bilgen, H., Cebeci, D., & Akman, I. (2005).Effect of foremilk and hindmilk on simple procedural pain in newborns. *Pediatrics international*, *47*, 252-257.

Valeri B. O., Holsti L.,& Linhares, M. B. M. (2015). Neonatal pain and developmental outcomes in children born preterm: a systematic review. *Clinical Journal of Pain*, *31*, 355-362.

van Dijk, M., de Boer, J. B., Koot, H. M., Tibboel, D., Passchier, J., & Duivenvoorden, H. J. (2000). The reliability and validity of the COMFORT scale as a postoperative pain instrument in 0 to 3-year-old infants. *Pain*, *84*, 367-377.

Vinall, J., Grunau, R. E., Brant, R., Chau, V., Poskitt, K. J., Synnes, A. R., & Miller, S. P. (2013). Slower postnatal growth is associated with delayed cerebral cortical maturation in preterm newborns. *Science Translational Medicine*, *5*, 168ra8-168ra8.

Vinall, J., Miller, S. P., Bjornson, B. H., Fitzpatrick, K. P., Poskitt, K. J., Brant, R., ... & Grunau, R. E. (2014). Invasive procedures in preterm children: brain and cognitive development at school age. *Pediatrics*, *133*, 412-421.

Walden, M., Hinson Penticoff, J., Stevens, B., Lotas, M. J., Kozinetz, C. A., Clark, A., & Avant, K. C. (2001).Maturational changes in physiologic and behavioral responses of preterm neonates to pain.*Advances in Neonatal Care, 1,* 94-106.

Weissman, A., Aranovitch, M., Blazer, S., & Zimmer, E. Z. (2009). Heel-lancing in newborns: behavioral and spectral analysis assessment of pain control methods. *Pediatrics*, *124*, e921-e926.

Weissman, A., Zimmer, E. Z., Aranovitch, M., & Blazer, S. (2012).Heart rate dynamics during acute pain in newborns. *European Journal of Physiology*, *464*, 593-599.

White, B. P., Gunnar, M. R., Larson, M. C., Donzella, B., & Barr, R. G. (2000).Behavioral and physiological responsivity, sleep, and patterns of daily cortisol production in infants with and without colic. *Child Development*, *71*, 862-877

Table 1. Study characteristics.

Study	N	Country	Gestational age	Postnatal age*	Acute pain procedure	Cardiovascular measure	Study design	Quality score^
Abad (2001)	15	Spain	37-42	< 4 days	Venipuncture	Mean HR	CS; Randomized trial	17
Altun-Koroglu (2010)	25	Turkey	37-41	4-8 days	Heel stick	Maximum HR	CS; Double-blind, placebo-controlled trial	13
Bilgen (2001)	34	Turkey	37-42	1-9 days	Heel stick	HR change (%)	CS; Randomized trial	14
Bucher (2000)	20	Switzerland	37-41	4 days	Heel stick	HR change (bpm)	CS; Randomized trial	14
Campos (1994)	20	United States	37-42	2 days	Heel stick	Mean HR	CS; Randomized trial	15
Cong (2012)	28	United States	28-32	< 14 days	Heel stick	HR increase, HRV	CS; Randomized cross-over trial	17
Cong (2009)	14	United States	30-32	< 9 days	Heel stick	Mean HR, HRV	CS; Randomized cross-over trial	17
Craig (1993)	56	Canada	25-27, 28-30, 31-33, 34-36, 37-42	< 8 days	Heel stick	Mean HR	CS; Observational	17
de Jesus (2011)	41	Brazil	37-41	< 2 days	Heel stick	HRV	CS; Observational	15
de Oliveira (2012)	36	Brazil	37-41	< 2 days	Heel stick	Maximum HR, HRV	CS; Observational	14
Gormally (2000)	21	Canada	37-42	2 days	Heel stick	Mean HR, HRV	CS; Randomized controlled trial	15
Goubet (2001)	14	United States	28-32	4 days, 21 days	Heel stick	HR change	C; Observational	13
Gray	15	United	37-42	< 3	Heel stick	Mean HR	CS;	17

(2000)		States		days			Randomized controlled trial	
Greenberg (2002)	21	United States	37-42	< 1 day	Heel stick	Mean HRV	CS; Randomized trial	13
Grunau (2010)	138	Canada	≤ 28, 29-32, 38-41	4 months	Immunization	Mean HR	C; Observational	16
Haouari (1995)	15	England	37-42	< 6 days	Heel stick	HR change (%)	CS; Double blind, placebo controlled trial	15
Jatana (2003)	25	India	37-42	< 7 days	Heel stick	HR change (bpm)	CS; Randomized trial	10
Johnston (2007)	20	Canada	32-35	< 10 days	Heel stick	Mean HR	CS; Randomized cross over trial	15
Johnston (1996)	89	Canada	27, 32	4 days, 5 weeks	Heel stick	Mean HR	CS; Observational	15
Kostandy (2013)	19	United States	37-42	1 day	Hepatitis B Vaccination	Mean HR	CS; Randomized controlled trial	17
Leite (2009)	29	Brazil	37-42	< 7 days	Heel stick	Mean HR	CS; Randomized clinical trial	17
Lindh (1999)	25	Sweden	37-42	4-5 days	Heel stick	Mean HR, HRV	CS; Observational	11
Lindh (2000)	28	Sweden	37-42	3 days	Venipuncture	Mean HR, HRV	CS; Randomized, double blind trial	15
Lindh (2003)	45	Sweden	37-42	3 months	DPT Vaccination	Mean HR, HRV	CS; Randomized, double blind, controlled trial	14
Lucas-Thompson (2008)	49	United States	28-31, 32-34	3-5 days, 3-5 weeks	Heel stick	Mean HR	C; Observational	17
Oberlander (2002)	23	Canada	37-42	2-3 days	Heel stick	Mean HR, HRV	CS; Observational	13
Oberland	22	Canada	37-42	2	Heel stick	Mean HR,	C;	13

er (2005)				months		HRV	Observational	
Oberlander (2002b)	12	Canada	24-28	27-54 days	Heel stick	Mean HR, HRV	CS; Observational	11
Ors (1999)	34	Turkey	37-42	< 9 days	Heel stick	HR change (%)	CS; Randomized trial	15
Owens (1984)	20	United States	37-42	2 days	Heel stick	Mean HR	CS; Observational	12
Sajedi (2006)	32	Iran	37-42	< 1 day	Intramuscular injection	Mean HR	CS; Randomized trial	13
Shibata (2013)	47	Japan	37-42	3-4 days	Heel stick	Mean HR	CS; Observational	14
Singh (2000)	150	India	32-34, 35-37, 37-42	< 7 days	Heel stick	Mean HR	CS; Observational	8
Stevens (1993)	40	Canada	32-34	< 5 days	Heel stick	Mean HR	CS; Descriptive	15
Stevens (1994)	124	Canada	32-34	≤ 5 days	Heel stick	Mean HR, Maximum HR, HRV	CS; Observational	17
Taksande (2005)	80	India	37-42	< 7 days	Venipuncture	Mean HR	CS; Observational	11
Upadhyay (2004)	41	India	37-42	<15 days	Venipuncture	Mean HR	CS; Randomized, placebo-controlled, double-blind trial	16
Uyan (2005)	21	Turkey	37-42	< 11 days	Heel stick	HR change (%), Maximum HR	CS; Randomized controlled trial	14
Walden (2001)	11	United States	24-26 weeks	21 days	Heel stick	Mean HR, Maximum HR	C; Quasi-experimental, repeated measures	18
Weissman (2009)	29	Israel	37-42	2-3 days	Heel stick	HR increase (bpm), HRV	CS; Randomized trial	11
Weissman (2012)	24	Israel	37-42	4-6 days	Heel stick	Mean HR, HRV	CS; Randomized controlled trial	10

Note. CS= Cross sectional study, C= Cohort study. CS and C Quality Scores are out of 20 and 21, respectively.

*Postnatal Age = Age at Measurement
^ out of 20 or 21 depending on research design

Table 2. Description of study covariates included in the cardiovascular analyses.

Study	Covariates
Abad (2001)	N/A
Altun-Koroglu (2010)	N/A
Bilgen (2001)	N/A
Bucher (2000)	Sex, nurse, number of lances needed, baseline heart rate and activity.
Campos (1994)	The number of additional sticks required to obtain the blood sample, the duration of the heel stick, the frequency of crying, and the average HR.
Cong (2012)	N/A
Cong (2009)	N/A
Craig (1993)	N/A
de Jesus (2011)	Gestational age, birth weight, sex, mode of delivery, diabetic mothers, breastfed one hour before puncture, received oral glucose.
de Oliveira (2012)	PIPP score in the period before the heel prick.
Gormally (2000)	Pre-intervention baseline (percentage of time crying in the last two minutes before beginning the interventions).
Goubet (2001)	N/A
Gray (2000)	N/A
Greenberg (2002)	Age, weight, time since last feeding, heel stick and blood collection procedure length, and gestational age.
Grunau (2010)	Corrected chronological age.
Haouari (1995)	N/A
Jatana (2003)	N/A
Johnston (2007)	Apgar scores at 5 minutes, gestational age at birth, time since last painful procedure, number of painful procedures since admission, or received

	indomethacin in the past 12 hours.
Johnston (1996)	Frequency of invasive procedures, severity of illness, ventilation status, sex.
Kostandy (2013)	N/A
Leite (2009)	N/A
Lindh (1999)	N/A
Lindh (2000)	N/A
Lindh (2003)	N/A
Lucas-Thompson (2008)	Number of prior heel sticks, duration of blood draws, sex, baseline heart rate.
Oberlander (2002)	Breast-fed, SSRI exposure, age at time of acute pain, maternal analgesia, dose of SSRI at delivery, dose of clonazepam at time of delivery.
Oberlander (2005)	Breast-fed, SSRI exposure, age at time of acute pain, maternal analgesia, dose of SSRI at delivery, dose of clonazepam at time of delivery.
Oberlander (2002b)	N/A
Ors (1999)	N/A
Owens (1984)	Sex.
Sajedi (2006)	Sex.
Shibata (2013)	N/A
Singh (2000)	N/A
Stevens (1993)	N/A
Stevens (1994)	N/A
Taksande (2005)	N/A
Upadhyay (2004)	N/A
Uyan (2005)	N/A
Walden (2001)	N/A

Weissman (2009)	N/A
Weissman (2012)	N/A

Note. Not applicable = N/A.

Table 3. Mean and standard deviations for heart rate response to acute pain at less than 7 postnatal days.

Gestational Age	Reference	Mean HR (bpm)	SD
25- 27 Weeks			
	Craig (1993)	172.38	17.22
28-32 Weeks			
	Cong (2009)	165.00	14.00
	Craig (1993)	168.20	10.50
	Craig (1993)	155.25	21.57
	Lucas-Thompson (2008)	169.27	10.89
32-34 Weeks			
	Singh (2000)	183.40	15.93
	Stevens (1994)	162.20	15.36
	Stevens (1993)	154.00	13.00
	Lucas-Thompson (2008)	158.18	15.19
34-37 Weeks			
	Craig (1993)	163.20	27.82
	Singh (2000)	165.30	16.50
37-42 Weeks			
	Abad (2001)*	170.00	N/A
	Craig (1993)	145.86	19.22
	Campos (1994)	174.00	16.60
	Gormally (2001)*	180.00	N/A

	Gray (2009)*	123.00	N/A
	Kostandy (2013)*	155.00	N/A
	Leite (2009)	172.70	21.50
	Lindh (1999)	134.00	19.00
	Lindh (2000)	144.00	20.00
	Oberlander (2002)*	168.00	N/A
	Shibata (2013)*	170.00	N/A

Note. * denotes numbers that were extrapolated from graphs.

Table 4. Mean and standard deviations for heart rate change from baseline in response to acute pain at less than 7 postnatal days.

Gestational Age	Reference	HR Change	SD
28-32 Weeks			
	Goubet (2001)*	0-15 bpm	N/A
37-42 Weeks			
	Altun-Korglu (2010)	37.00%	N/A
	Bilgen (2001)	19.00%	N/A
	Bucher (2000)*	45 bpm	N/A
	Gray (2000)	36-38 bpm	N/A
	Haoari (1995)	11.40%	3.0
	Jatana (2003)	31.48 bpm	6.66 bpm
	Ors (1995)	19.00%	N/A
	Owens (1984)	49.00 bpm	17.5 bpm
	Sajedi (2006)	10.81	N/A
	Uyan (2005)	38.20%	N/A
	Weissman (2009)	36.50 bpm	19.50 bpm

Note. * denotes numbers that were extrapolated from graphs.

Table 5. Mean and standard deviations for low frequency heart rate variability in response to acute pain at less than 7 postnatal days.

Gestational Age	Reference	Mean LF HRV	SD
28-32 Weeks			
	Cong 2009	17.62	24.55
37-42 Weeks			
	Gormally (2001)*	1.65	N/A
	Lindh (1999)	4.2	0.4
	Lindh (2000)	4.00	0.39
	Oberlander (2002)*	11.0	N/A
	Weissman (2012)	1.45	0.38

Note. Heart rate variability = HRV, low frequency = LF, standard deviation = SD.

Table 6. Mean and standard deviations for high frequency heart rate variability in response to acute pain at less than 7 postnatal days.

Gestational Age	Reference	Mean HF HRV	SD
28-32 Weeks			
	Cong (2009)	23.52	35.96
37-42 Weeks			
	de Oliveira (2012)	.44	.69
	Greenberg (2002)*	2.5	N/A
	Lindh (1999)	3.4	0.60
	Lindh (2000)	3.23	0.45
	Oberlander (2002)*	2.0	N/A
	Weissman (2012)	0.76	0.50

Note. Heart rate variability = HRV, high frequency = HF, standard deviation = SD.

Table 7. Mean and standard deviations for low frequency/high frequency ratio in response to acute pain at less than 7 postnatal days.

Gestational Age	Reference	Mean LF/HF Ratio	SD
28-32 Weeks			
	Cong (2009)	1.75	1.84
37-42 Weeks			
	Oberlander (2002)*	6.00	N/A
	Weissman (2012)	6.1	3.2

Note. Low frequency = LF, high frequency = HF, standard deviation = SD.

Table 8. Mean and standard deviations for total heart rate variability in response to acute pain at less than 7 postnatal days.

Gestational Age	Reference	Mean Total HRV	SD
32-34 Weeks			
	Stevens (1994)	4.52	2.95
37-42 Weeks			
	Lindh (1999)	4.30	0.40
	Lindh (2000)	4.10	0.35

Note. Heart rate variability = HRV, standard deviation = SD.

Table 9. Mean and standard deviations for maximum heart rate in response to acute pain at less than 7 postnatal days.

Gestational Age	Reference	Maximum HR (bpm)	SD
37-42 Weeks			
	Campos (1994)	192.00	11.80
	de Jesus (2011)	149.00	N/A
	Owens (1984)	179.40	13.40
	Singh (2000)	160.30	20.00
	Taksande (2005)	151.00	10.40
	Uyan (2005)	186.00	N/A

Note. Beats per minute = bpm, heart rate = HR, standard deviation = SD.

Table 10. Confidence limits, pooled means and standard deviations for mean heart rate at less than 7 postnatal days based on gestational age.

Gestational Age (wks)	Studies (n)	Lower 95% CL	Upper 95% CL	Mean	SD
28-32	4	162.52	172.9	167.71	0.62
32-34	4	147.68	176.64	162.16	3
34-37	2	150.26	178.9	164.58	0.36
37-42	7	142.45	167.25	154.85	3.10

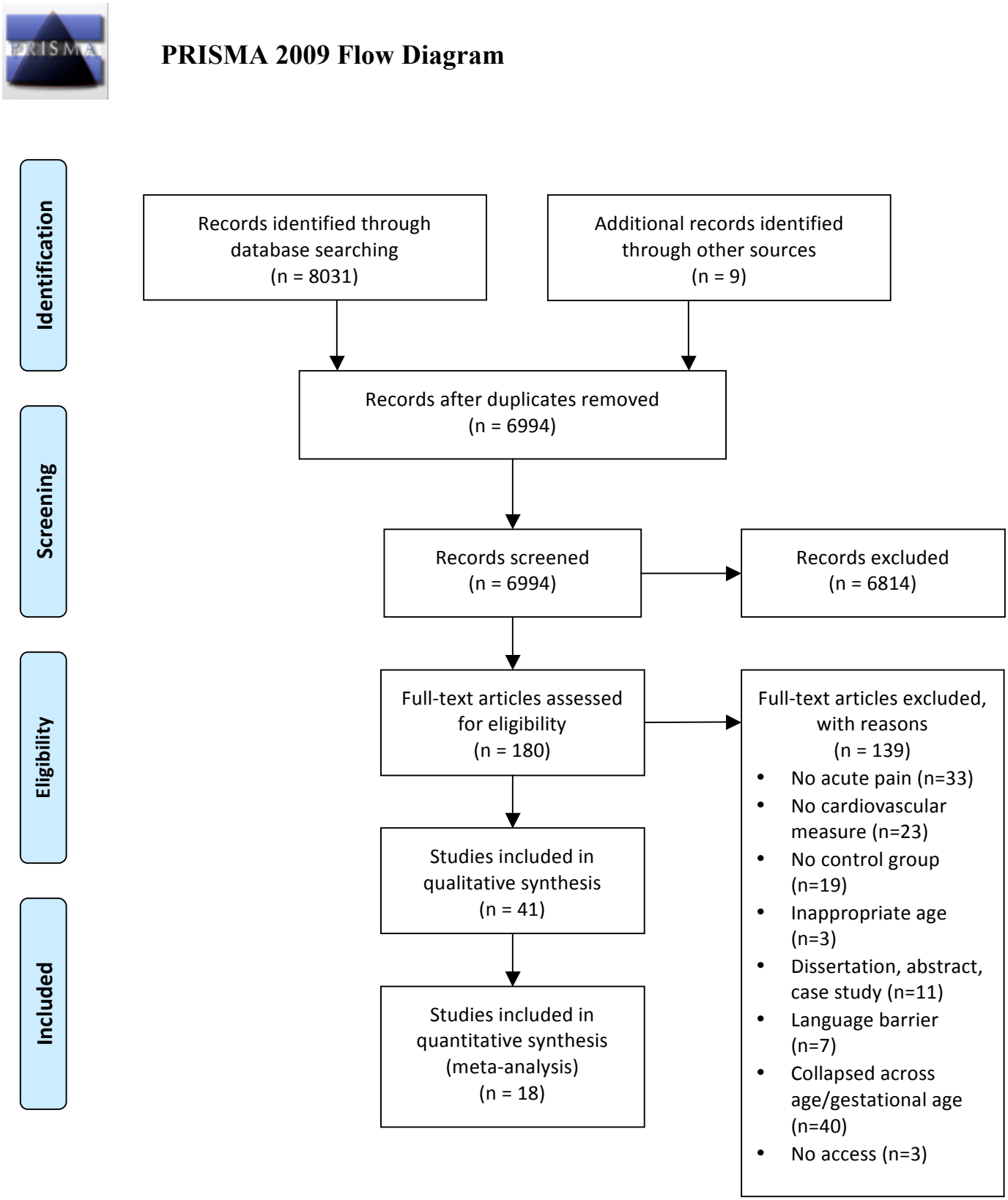
Note. Confidence limit = CL, standard deviation = SD. Heart rate reported in beats per minute (bpm).

Table 11. Timing of Mean HR, HR change, and HRV calculations.

Study	Time of measurement
Abad (2001)	0-120 seconds post-needle procedure.
Altun-Koroglu (2010)	Difference between baseline and maximum HR.
Bilgen (2001)	0-60 seconds post-needle procedure.
Bucher (2000)	Difference between baseline and maximum HR.
Campos (1994)	0-15 seconds post-needle procedure.
Cong (2012)	0-30 seconds post-needle procedure.
Cong (2009)	0-15 seconds post-needle procedure.
Craig (1993)	0-10 seconds post-needle procedure.
de Jesus (2011)	0-180 seconds post-needle procedure.
de Oliveira (2012)	15 seconds pre-needle to 30 seconds post-needle procedure.
Gormally (2000)	0-60 seconds post-needle procedure.
Goubet (2001)	0-30 seconds post-needle procedure.
Gray (2000)	0-10 seconds post-needle procedure.
Greenberg (2002)	0-300 seconds post-needle procedure.
Grunau (2010)	0-30 seconds post-needle procedure.
Haouari (1995)	Difference between baseline and maximum HR.
Jatana (2003)	Difference between baseline and maximum HR.
Johnston (2007)	0-30 seconds post-needle procedure.
Johnston (1996)	0-15 seconds post-needle procedure.
Kostandy (2013)	0-60 seconds post-needle procedure.

Leite (2009)	0-250 seconds post-needle procedure
Lindh (1999)	0-40 seconds post-needle procedure.
Lindh (2000)	0-80 seconds post-needle procedure.
Lindh (2003)	0-45 seconds post-needle procedure.
Lucas-Thompson (2008)	0-30 seconds post-needle procedure.
Oberlander (2002)	20-160 seconds post-needle procedure.
Oberlander (2005)	20-160 seconds post-needle procedure.
Oberlander (2002b)	20-160 seconds post-needle procedure.
Ors (1999)	Difference between baseline and maximum HR.
Owens (1984)	0-15 seconds post-needle procedure.
Sajedi (2006)	Difference between mean HR before and after needle procedure.
Shibata (2013)	0-2 seconds post-needle procedure.
Singh (2000)	Maximal change post-needle procedure.
Stevens (1993)	0-15 seconds post-needle procedure.
Stevens (1994)	0-15 seconds post-needle procedure.
Taksande (2005)	Maximal change post-needle procedure.
Upadhyay (2004)	0-60 seconds post-needle procedure.
Uyan (2005)	0-60 seconds post-needle procedure.
Walden (2001)	0-30 seconds post-needle procedure.
Weissman (2009)	10-130 seconds post-needle procedure.
Weissman (2012)	10-130 seconds post-needle procedure.

Figure 1. Included study flow chart following PRISMA guidelines.



APPENDIX A

Medline Search Strategy

1. Acute pains/
2. acute pain*.mp.
3. (bloodsampl* or immuni* or inoculat* or vaccin* or inject* or "finger prick*" or finger-prick or "heel prick*" or heel-prick* or "heel lance*" or heel-lance* or "heel puncture*" or heel-puncture* or "heel stick" or suture* or (laceration* adj3 repair*)).mp.
4. ("lumbar puncture" or lumbar-puncture* or "spinal tap*" or spinal-tap*).mp.
5. ("bone marrow aspiration" or "bone marrow biops*").mp.
6. (intravenous or intra-venous or venepuncture* or venipuncture* or venous cannulation* or (arterial blood gas* and cannul*)).mp.
7. ((catheter adj6 insert*) or catheter* or port-a-cath* or portacath).mp.
8. ("central line" adj6 (insert* or remov*)).mp.
9. (central venous catheter* adj6 insert*).mp.
10. (localanalges* or local anaesthe* or local anesthe*).mp.
11. ((arterial puncture or artery) adj6 puncture*).mp.
12. "arterial line*".mp.
13. (thoracocentesis or paracentesis).mp.
14. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13
15. exp Pain/
16. Pain Measurement/
17. PAIN THRESHOLD/
18. pain*.mp.
19. 15 or 16 or 17 or 18
20. 14 and 19
21. ((vaccin* adj6 pain) or (cannul* adj6 pain) or (acute pain* adj6 pain*) or (procedure* adj6 pain*) or (procedure-related adj6 pain)).mp.
22. 20 or 21
23. Child, Preschool/
24. exp Infant/
25. (baby or babies or neonate* or newborn or child* or infant* or paediatric* or pediatric*).mp.
26. 23 or 24 or 25
27. 22 and 26
28. Heart Rate/
29. (physiology or physiological).mp.
30. heart rate variability.mp.
31. Psychophysics/
32. Autonomic Nervous System/
33. vagal tone.mp.
34. Electrocardiograph*.mp.
35. low frequency.mp.
36. high frequency.mp.
37. (biobehaviour or biobehavior).mp.
38. respiratory sinus arrhythmia.mp.

39. respirat*.mp.
40. (spectral analysis or spectrum analysis).mp.
41. (frequency domain measures or frequency domain analysis).mp.
42. Arterial Pressure/
43. 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42
44. 27 and 43

APPENDIX B

Quality Assessment Measure

1. Is the hypothesis/aim/objective of the study clearly described?
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?
3. Is the design of the study described?
4. Is the setting of the study described?
5. Is the source of the subjects studied stated?
6. Is the distribution of the study population by age described?
7. Is the distribution of the study population by gender described?
8. Is the sample size stated?
9. Is the participation/follow up described?
10. Are non-participants/subjects lost to follow up described?
11. Are the main findings of the study clearly described?
12. Are the statistical methods described?
13. Have actual probability values been reported (e.g., 0.035 rather than < 0.05) for the main outcomes except where the probability value is less than 0.001?
14. Are confidence intervals/standard deviations given?
15. Are any conclusions stated?
16. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?
17. Were the subjects who were prepared to participate in the study representative of the entire population from which they were recruited?
18. Was the participation/follow-up rate > 80%?
19. Were the main outcome measures used accurate (valid and reliable)?
20. Was the sample size justified?
21. Analysis adjusts for length of follow up?