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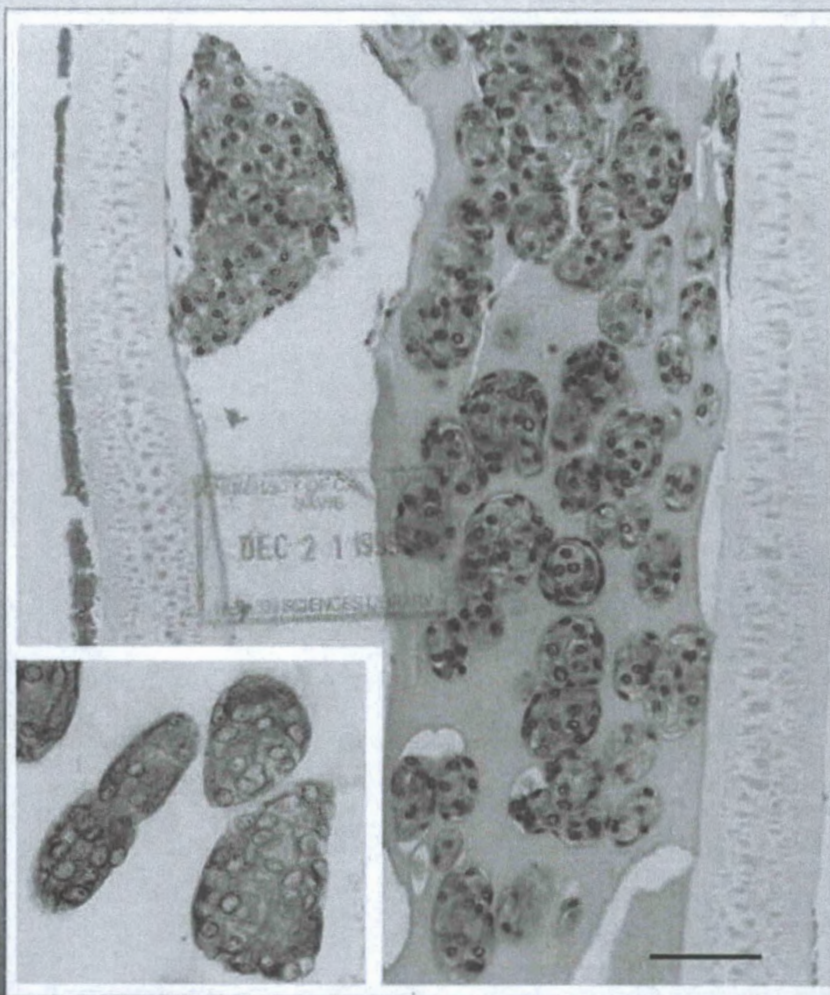
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CONTENTS

Publisher's note

vii

REVIEW ARTICLE

- Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action
S.H. Sindrup, T.S. Jensen (Denmark) 389

RESEARCH PAPERS

- Chronic thalamotomy increases pain-related behavior in rats
N.E. Saadé, A.I. Kafrouni, C.Y. Saab, S.F. Atweh, S.J. Jabbur (Lebanon) 401
- Effects of naloxone on nitrous oxide actions in healthy volunteers
J.P. Zacny, A. Conran, H. Pardo, D.W. Coalson, M. Black, P.A. Klock, J.M. Klafta (USA) 411
- The relationship of sex and clinical pain to experimental pain responses
R.B. Fillingim, R.R. Edwards, T. Powell (USA) 419
- Hypoalgesia elicited by a conditioned stimulus is blocked by a μ , but not a δ or a κ , opioid antagonist injected into the rostral ventromedial medulla
H. Foo, F.J. Helmstetter (Australia, USA) 427
- Combined systemic administration of the glycine/NMDA receptor antagonist, (+)-HA966 and morphine attenuates pain-related behaviour in a rat model of trigeminal neuropathic pain
D. Christensen, M. Gautron, G. Guilbaud, V. Kayser (France) 433
- Modulation of an inhibitory reflex in single motor units in human masseter by tonic painful stimulation
P. Svensson, A.S. McMillan, T. Graven-Nielsen, K. Wang, L. Arendt-Nielsen (Denmark, Hong Kong) 441
- Effects of combined hemotoxic and anterolateral spinal lesions on nociceptive sensitivity
C.J. Vierck Jr, A.R. Light (USA) 447
- A PET activation study of dynamic mechanical allodynia in patients with mononeuropathy
P. Petrovic, M. Ingvar, S. Stone-Elander, K.M. Petersson, P. Hansson (Sweden) 459
- Effects of a brief and economical intervention in preparing patients for surgery: does coping style matter?
J. Miró, R.M. Raich (Spain) 471
- Dynamic adjustments of walking behavior dependent on noxious input in experimental low back pain
R. Moe-Nilssen, A.E. Ljunggren, E. Torebjörk (Norway, Sweden) 477
- Spatio-temporal pattern of induction of bradykinin receptors and inflammation in rat dorsal root ganglia after unilateral nerve ligation
A. Eckert, G. Segond von Banchet, S. Soppe, M. Petersen (Germany) 487
- Pain perception, somatosensory event-related potentials and skin conductance responses to painful stimuli in high, mid, and low hypnotizable subjects: effects of differential pain reduction strategies
V. De Pascalis, M.R. Magurano, A. Bellusci (Italy) 499
- Lack of cross-tolerance between the antinociceptive effects of systemic morphine and asimadoline, a peripherally-selective κ -opioid agonist, in CCI-neuropathic rats
J. Walker, G. Catheline, G. Guilbaud, V. Kayser (Australia, France) 509
- Dorsal horn NMDA receptor function is changed after peripheral inflammation
F. Svendsen, L.J. Rygh, K. Hole, A. Tjølsen (Norway) 517
- Intraarticular morphine versus dexamethasone in chronic arthritis
A. Stein, A. Yassouridis, C. Szopko, K. Helmke, C. Stein (Germany) 525
- Accuracy of recall of usual pain intensity in back pain patients
J.E. Bolton (UK) 533
- Plasma levels of substance P, neuropeptide Y and vasoactive intestinal polypeptide in patients with chronic tension-type headache
M. Ashina, L. Bendtsen, R. Jensen, R. Ekmann, J. Olesen (Denmark, Sweden) 541
- Occlusal treatments in temporomandibular disorders: a qualitative systematic review of randomized controlled trials
H. Forssell, E. Kalso, P. Koskela, R. Vehmanen, P. Puukka, P. Alanen (Finland) 549
- Improving the efficiency of the formalin test
F.V. Abbott, R. Ocvirk, R. Najafee, K.B.J. Franklin (Canada) 561
- The three dimensions of headache impact: pain, disability and affective distress
K.A. Holroyd, P. Malinoski, M.K. Davis, G.L. Lipchik (USA) 571
- Brief and prolonged effects of Lissauer tract stimulation on dorsal horn cells
P.D. Wall, M. Lidierth, P. Hillman (UK, Israel) 579



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Plasticity of sodium channel expression in DRG neurons in the chronic constriction injury model of neuropathic pain S.D. Dib-Hajj, J. Fjell, T.R. Cummins, Z. Zheng, K. Fried, R. LaMotte, J.A. Black, S.G. Waxman (USA, Sweden)	591
Everyday life with osteoarthritis or rheumatoid arthritis: independent effects of disease and gender on daily pain, mood, and coping G. Affleck, H. Tennen, F.J. Keefe, J.C. Lefebvre, S. Kashikar-Zuck, K. Wright, K. Starr, D.S. Caldwell (USA)	601
Suckling- and sucrose-induced analgesia in human newborns E.M. Blass, L.B. Watt (USA)	611
CLINICAL NOTES	
Pain relief of oral ulcer by dibucaine-film K. Yamamura, T. Yotsuyanagi, T. Okamoto, T. Nabeshima (Japan)	625
Diabetic neuropathic pain in a leg amputated 44 years previously S.M. Rajbhandari, J.A. Jarratt, P.D. Griffiths, J.D. Ward (UK)	627
Effects of intravenous methylnaltrexone on opioid-induced gut motility and transit time changes in subjects receiving chronic methadone therapy: a pilot study C.S. Yuan, J.F. Foss, M. O'Connor, J. Osinski, M.F. Roizen, J. Moss (USA)	631
Author Index to Volume 83	637
Contents to Volume 83	641



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Suckling- and sucrose-induced analgesia in human newborns

Elliott M. Blass^{a, b,*}, Lisa B. Watt^b

^aDepartment of Psychology, University of Massachusetts, Amherst, MA, USA

^bDepartment of Pediatrics, Boston Medical Center, 91 E. Concord Street, Maternity 6, Boston, MA 02118, USA

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Abstract

This experiment had three goals: 1. To identify the basis of sucking-induced analgesia in healthy, term, newborn humans undergoing the painful, routine, procedure of heel lance and blood collection. 2. To evaluate how taste-induced and sucking-induced analgesias combine to combat pain. 3. To determine whether facial grimacing was an accurate index of diminished pain, or whether it was linked to tissue trauma. We report that: 1. Sucking an unflavored pacifier was analgesic when and only when suck rate exceeded 30 sucks/min. 2. The combination of sucrose and nonnutritive sucking was remarkably analgesic; we saw no behavioral indication in nine of the ten infants that the heel lance had even occurred. 3. Grimacing was reduced to almost naught by procedures that essentially eliminated crying and markedly reduced heart rate during the blood harvesting procedure. © 1999 International Association for the Study of Pain. Published by Elsevier Science B.V.

Keywords: Human newborns; Pain; Heel lance; Suckling; Sucrose; Pacifier; Crying; Grimacing; Heart rate

1. Introduction

Suckling-induced analgesia (SIA) is of current theoretical and applied interest in pediatrics and developmental neurobiology (Blass, 1996; Stevens et al., 1997; Barr et al., 1999; Blass et al., 1999a). Causal relationships have been established between suckling, a noninvasive and nonpharmacological treatment, and pain prevention (Field and Goldson, 1984; Gunnar et al., 1984; Blass and Hoffmeyer, 1991; Campos, 1994; Blass and Shah, 1995; Barr et al., 1998) and some underlying mechanisms have been identified (Blass and Fitzgerald, 1988; Blass et al., 1987; Ren and Blass, 1997). The two identified components of SIA utilize separate detection systems and neural mechanisms to reach a final common analgesia pathway. The orogustatory component has been engaged in both rat (Blass and Fitzgerald, 1988; Shide and Blass, 1989) and full term (Blass and Hoffmeyer, 1991; Blass and Ciaramitaro, 1994; Blass and Shah, 1995; Barr et al., 1998) and premature, human infants (Bucher et al., 1995; Abad et al., 1996; Ramenghi et al., 1996a,b; Skogsdal et al., 1997) by sweet tastes and milk flavor (Blass, 1997a,b). Nonnutritive sucking of either a pacifier or a nonlactating nipple causes analgesia in both human and rat full term newborns through stimulation of oropharyngeal tactile- and mechanoreceptors (Smith et al.,

1990; Blass et al., 1995). The analgesic qualities of nonnutritive sucking in premature infants have not yet been assessed (see Stevens et al., 1997 for meta-analysis).

Taste-induced analgesia in both human (Blass et al., 1989; Smith et al., 1990) and rat (Blass and Fitzgerald, 1988; Shide and Blass, 1989) newborns endures well after ingestion has been completed. It is mediated by opioid mechanisms: it does not survive naloxone or naltrexone blockade in rats (Blass and Fitzgerald, 1988; Shide and Blass, 1989; Blass and Shide, 1994) and does not appear in infants born to women who were maintained on methadone during pregnancy (Blass and Ciaramitaro, 1994). In contrast, orotactile induced calming and analgesia in rat and human infants terminates almost immediately upon sucking completion and is not opioid mediated (Blass and Ciaramitaro, 1994). It is not blocked by high doses of naloxone in rats and sucking very effectively calmed infants with a prenatal methadone history who were not calmed by sweet taste (Blass and Ciaramitaro, 1994).

In 10-day-old rats, sucking the nonlactating nipples of anesthetized females trebles escape latencies from a 48°C heat probe applied to ear or shoulder (Blass, 1996). Combining nonnutritive sucking (NNS) with sweet taste is even more protective (Ren and Blass, 1997). Orotactile influences on analgesia in human infants have been evaluated in only four studies, however. NNS reduces crying during circumcision by about 30% (Blass and Hoffmeyer, 1991; Gunnar et al., 1984) and sucking a sweet pacifier caused a

* Corresponding author. Tel.: +1-617-414-7906; fax: +1-617-414-7297.

E-mail address: ebllass@medusa.sbs.umass.edu (E.M. Blass).

70% reduction in crying (Blass and Hoffmeyer, 1991), thereby demonstrating intermodal potentiation of pain relief. Heart rate elevations were far more modest in infants who received a sucrose flavored pacifier than in control infants who were not treated during circumcision. The magnitude of the sucrose-induced changes was equivalent to those infants who had been given a Dorsal Penile Nerve Block (DPNB) prior to circumcision. Oxygen saturation measures were consistent (Herschel et al., 1998). The effects of NNS on pain perception during and following the less traumatic, but more fully studied procedure, of blood collection via heel lance has been assessed only twice (Field and Goldson, 1984; Campos, 1994). Because more sensitive behavioral assessments of analgesia are now well established (Grunau and Craig, 1987; Craig et al., 1993) and because the effects of combining orotactile and orogustatory stimulation have not been adequately determined in any species, the current study closely scrutinizes the relationship between parameters of NNS and the analgesia that it induces. This reflects our view that identifying the microstructure of a relationship will provide guidance towards revealing the underlying mechanism. To determine how intermodal pain reducing treatments interact, we combined suprathreshold levels of sucking and sweet taste prior to and during a standard heel lance procedure.

Accordingly, the present report focuses on three issues concerning pain alleviation in newborns. Firstly, through close study of individual infants during and following the heel lance procedure, we sought to identify some of the circumstances under which NNS alone alleviated pain.¹ Secondly, by allowing newborns to suck a sweetened pacifier before and during heel lance, we determined whether orotactile (sucking) and orosensory (sucrose) afferents combined to further reduce pain reactivity. This approach provides parallels with animal findings to help reveal underlying pain relief mechanisms. The third goal of this study was to evaluate grimacing as an index of pain reduction. If grimacing is tightly linked to tissue trauma, and not to pain perception, then it should *not* diminish in situations in which crying, and possibly heart rate are reduced in humans, and in which pain threshold is markedly increased in rats. However, if grimacing is a valid indicator of pain perception, as asserted by Grunau and colleagues (Grunau and Craig, 1987; Craig et al., 1993), then it too should be correspondingly reduced by interventions that reduce crying during heel lance.

¹ Although the distinction between pain and pain behaviors is important, no objective criterion to differentiate the two can be used in newborns. We have been persuaded by the arguments of Grunau and Craig (1987) and their colleagues Craig et al. (1993) on this issue. The current measures, accordingly, are thought to reflect pain.

2. Materials and methods

2.1. Subjects

Forty full term infants newly born at Boston Medical Center, in Boston, MA were randomly assigned to one of four experimental groups ($n = 10$ infants/group) representing the treatments of sucrose alone, water alone, a pacifier dipped in water or one dipped in sucrose. The effects of these treatments were assessed through crying, grimacing and heart rate responses during and following mandatory heel stick screening for PKU (now known as the Newborn Screening Program Blood Test-NSPBT) prior to well-nursery discharge. Data were collected between June 1997 and June 1998.

Four of the newborns were delivered by cesarean section and 36 by normal vaginal delivery. Twenty-two infants were African American, nine White, five Hispanic, one Asian, one American Indian and two infants were not classified racially. Twenty-three infants were female. The birth weight range was between 2.4 and 4.2 kg.; mean 3.3 kg. Apgar scores were 7 or above at 1 or 5 min. Infants were between 34 and 55 h old at the time of testing, which generally began between 07:00 and 08:00 h. None of these indices differed statistically among groups. A single phlebotomist performed 36 of the heel sticks, thereby minimizing variability.

Seventeen infants were exclusively breastfed and were essentially equally distributed among the four study conditions. The last feeding, bottle or breast, had occurred between 30 min and 4 h prior to the study. None of the infants enrolled presented any evidence of congenital anomalies or medical complications. No infant required either oxygen administration or ventilatory support. This was the first heel stick for 32 of the infants. The remaining eight infants were essentially equally distributed among the four groups and their behaviors were indistinguishable from those of infants for whom this was the initial stick. Ten infants had been circumcised the day prior to the study. Their distribution among the four groups was also essentially equal.

Written informed consent was obtained from a parent, almost always the mother, on the afternoon preceding the study. Ninety-four percent of the parents approached agreed to allow their infants to participate. The institutional review boards at the Boston Medical Center and University of Massachusetts approved the protocol and the informed consent forms.

2.2. Data collection

An infant was assigned to a group by random selection on the morning of the study. The infant was moved to a quiet room adjacent to the newborn nursery where it lay supine in his or her crib. A mounted video camera (Sony Model CCD-TRV32) recorded crying and facial grimacing continuously.

Heart rate was read aloud off a monitor every 10 s as beats/min (BPM). This form of data collection for heart rate is extremely convenient and, as will be seen, captures both the magnitude and duration of change caused by the painful procedure. The entire experiment required about 8–10 min.

2.3. Detailed procedure

The experimental protocol is presented as a time line in Fig. 1. At about 4 min before blood collection a warming pad was wrapped around the infant's foot and three safety electrodes (Klear Trace 2000-S) were placed on the thoracic region for heart rate recording. The infant was again wrapped in a receiving blanket and returned supine to his or her bassinet. The video camera was adjusted to an enface position with the infant, and video quality was monitored via closed circuit TV to ensure facial clarity. Baseline data for all three measures were obtained for 60 s at which time water or a 12% sucrose solution (5.65 g sucrose/48 ml sterile water) was delivered by syringe or pacifier. The infant either swallowed 2-ml of solution delivered slowly over 2 min by syringe or sucked a pacifier (Mam Mini Mam orthodontic pacifier) dipped in the assigned solution every 30 s for the 2 min treatment period.

The blood collection process was then immediately initiated by removing the warming pad from the infant's foot, palpating the heel and lancing it. Blood was drawn for the standard newborn-screening test by the experienced phlebotomist who was not informed of either the purpose of the study or the infant's experimental condition. Each infant received only a single heel stick for blood collection. Collection time ranged from 1 to 3 min; mean collection time was 1 min 47 s. Blood collection started operationally with the heel lance and ended with the application of a Band-Aid. Measurements continued for the following 3 min (Recovery in Fig. 1).

2.4. Data analysis

2.4.1. Initial evaluation

Evaluators uninformed of experimental treatment scored the audio-videotapes continuously for crying and grimacing from the beginning of the 1-min baseline period to the end of the 3-min recovery period. It was impossible, of course, to blind scorers to pacifier vs. syringe deliveries. However,

they did not know which solution was tasted. Crying was defined as the presence of audible crying vocalizations. For grimacing, we followed the definitions of Grunau and Craig (1987) of a furrowing of the brow and eye squinting. These were reported to be the most reliable measures and were especially appropriate for the present study in which 50% of the infants sucked a pacifier during heel lance, thereby precluding using other facial expressions as an index of pain. Heart rate was transcribed every 10 s from the audio portion of the tape.

2.4.2. Second evaluation

As will be seen below, there was considerable variability in the incidence of crying and grimacing during heel lance of infants sucking a water-dipped pacifier. Accordingly we sought to establish a linkage between infant sucking and pain reactivity. The evaluation was conducted without knowledge of behavior during heel lance by scoring only that portion of an infant's video record, during the Treatment period, in which they sucked a pacifier. Each sucking movement, defined as a pacifier bob or, when the pacifier was occluded, a flexion of cheek and neck muscles that typify sucking was recorded in real time and a graphic representation for each infant was created. This record was then first analyzed for the number of individual sucks that occurred per min prior to heel lance. We then determined the length of each sucking bout, the number of sucking movements per bout and suck rate during the bout. Following all of these analyses, we returned to the videotapes to document crying in relation to sucking during and following heel lance without knowledge of behavior prior to lance. At this point, the same analysis of suck rate was undertaken to evaluate changes in rate that may have been induced by the lance procedure. Crying and grimacing were recorded in separate passes and a composite chart for each infant was generated to allow visualization of relationships between sucking, crying, and grimacing.

2.5. Statistical analyses

Outcome measures were derived from the scored videotapes and were expressed as changes in heart rate (HR) from treatment values, percentage of time crying, and percentage of time grimacing during the Heel Lance and Recovery periods. Each infant contributed one data point during

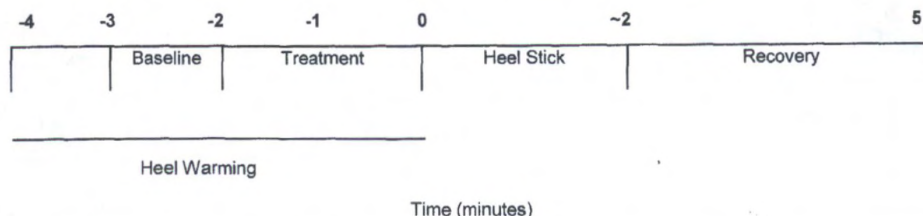


Fig. 1. Time line depicting experimental protocol used in this study and terminology used to represent time periods. The experiment started 4 min before blood collection, when a heel warmer was placed on the infant's foot, and ended 3 min after the termination of blood collection.

blood collection for crying and one for grimacing. Separate, between group, ANOVAs evaluated the statistical reliability of group differences. Particular differences were evaluated via planned paired-comparisons. Heart rate differences were determined via repeated measures ANOVA among the groups. A repeated measure ANOVA was also applied to each dependent variable for the recovery period. For crying and grimacing, each infant contributed a total of six data points for each measure, one for every 30-sec period.

3. Results

3.1. Overview

Two new findings of theoretical and clinical significance have been obtained and the utility of facial grimacing has been extended to include pain diminution. Firstly, sucking a pacifier was analgesic during blood collection as judged by lack of crying and facial grimacing when and only when an infant sucked at a rate that exceeded 32 sucks/min during the Treatment period that preceded heel lance. Secondly, sucking a sweetened pacifier had greater antinociceptive effect, as judged by grimacing, than did either sucking alone or tasting sucrose alone. In addition, sucrose alone and on a pacifier very much blunted HR elevation during and following the lance. The detailed analyses now follow.

Fig. 2 summarizes group effects for crying and facial grimacing during the time of blood collection. It is immediately clear that relative to the water-control and water-pacifier groups, sucrose in combination with a pacifier, drastically reduced crying and grimacing during the painful procedure of heel lance. According to ANOVAs for crying and for grimacing measures, statistically reliable main effects were obtained for the blood collection phase of the study. Posthoc comparisons showed that this held between water and both sucrose treatments for crying and grimacing.

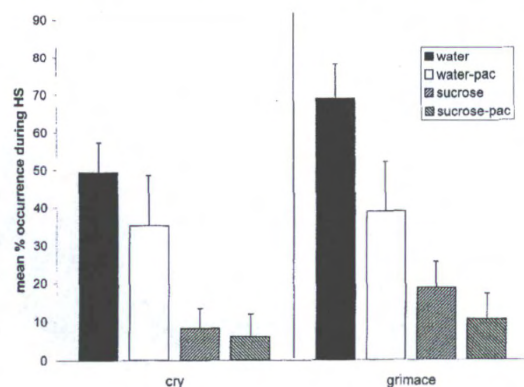


Fig. 2. Mean percentage of time ($\pm$ SEM) spent crying (left panel) and grimacing during blood collection of infants who had received either 2 ml of water or sucrose or a plain or sucrose-dipped pacifier over a 2-min delivery period prior to heel stick ($n = 10$ infants/group).

Sucrose alone reliably diminished both crying and grimacing relative to water controls ($P = 0.003$ and $P = 0.0003$, respectively). The differences relative to sucking a plain pacifier were statistically reliable for crying ($P = 0.02$) but not for grimacing ($P = 0.17$). Sucking a sweetened pacifier substantially reduced crying and grimacing relative to water alone and pacifier alone ($P = 0.002$, 0.002 and $P = 0.001$, 0.04 , respectively). Sucking a water dipped pacifier caused modest and variable reductions. The variability in the behavior in infants sucking a water pacifier is reflected in a tendency towards statistical reliability for grimacing, $P = 0.08$, although not for crying, $P = 0.38$ between water and water-pacifier groups during blood collection. This can not be attributed to baseline differences among groups because one-way ANOVAs on the data sets during the baseline period that preceded pacifier administration did not yield statistically reliable differences on any of the three measures.

Differences among groups were not statistically reliable during the 3-min recovery period. This reflects the fact that at least four of the infants in each of the water groups stopped crying and grimacing within seconds after the completion of blood collection. Crying and grimacing in the remaining infants was protracted. It is possible, of course, that this pattern of results would attain statistical reliability if a larger n was employed. The import of the pattern, in our view, however, is that an excellent phlebotomist reduces pain that is experienced during and following the blood collection procedure. Thus, pain levels drop below threshold during recovery, according to our measures.

Fig. 3, shows the first level of our finer grained post-hoc analysis by presenting the relationships between sucking, crying, grimacing, and change in heart rate individually for each infant who sucked a water-flavored pacifier. There was remarkable agreement between suck rate during Treatment prior to blood harvesting and pain responsivity during collection as indexed by all three measures. Infants whose suck rate was lower than 30-times/min prior to collection were not at all protected by pacifier sucking. The transition between protection and no-protection was essentially a step function. The infant whose suck rate was 32/min cried and grimaced 40% of the collection time. Moreover, the function on either side of the '30' divide was flat. Those with higher rates, presented very little pain related symptoms as measured here, and those who were on the left side of the divide were essentially indistinguishable from control infants who received water alone. The orderliness of these findings was not expected. We suggest that central antinociceptive mechanisms are engaged above a certain sucking frequency, although we have not ruled out other potentially causative cofactors, such as the strength of the negative pressure exerted by sucking.

The insert in Fig. 3 summarizes crying and grimacing in infants whose suck rates were below and above 30/min. Mean differences between groups are substantial. More importantly, the substantial variability observed in Fig. 3,

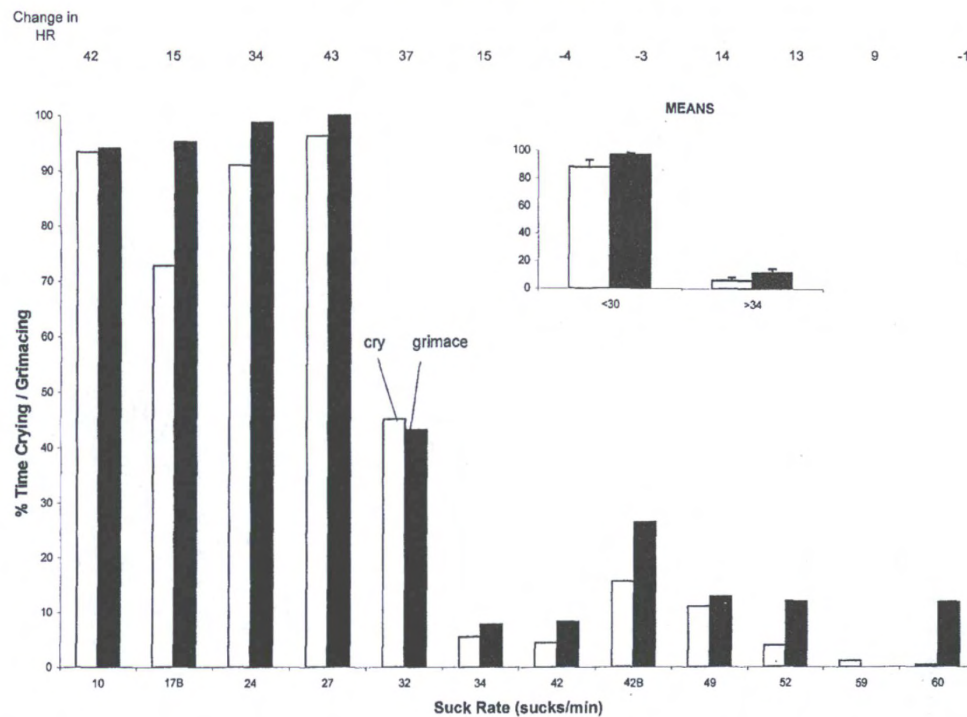


Fig. 3. Percentage of time spent crying and grimacing for individuals who sucked a water-dipped pacifier. Data are presented serially in order of suck rate prior to heel lance. Change in mean heart rate during heel lance and blood collection is indicated above each infant's cry and grimace scores. The enclosed figure represents the mean ($\pm$ SEM) crying and grimacing values of the two groups established by suck rate.

in which the standard errors were almost 45% of the mean value, vanished when infants were grouped according to suck rate. These differences were significantly reliable according to a *t*-test. ($P = 0.001$ for crying and $P = 0.0001$ for grimacing).

Although, the number of sucks per minute provides a convenient and easily conveyed metric for discriminating between successful and unsuccessful suckling intervention, it does not get at the underlying mechanism which probably reflects integration on a much smaller time scale. A micro-analysis of the relation between actual sucking and crying patterns in real time is presented in Fig. 4 for three representative newborns, one each with high, medium, or low rates of sucking. The infant with the highest suck rate during treatment occupies the uppermost tier. Numbers on the left side of each couplet reflect suck rate/minute prior to heel lance and on the right side, during and following lance.

A number of points are noteworthy. Firstly, all 'unprotected' infants in the water pacifier group emitted very few sucks per burst during the Treatment period. In fact, the highest number of sucks/individual burst for the 'unprotected' infants was 7 sucks/burst. This may be contrasted with the 'protected' infants whose mean number of sucks/burst often exceeded 20. Lack of crying can not be attributed to physical incompatibility of having a pacifier firmly seated

in one's mouth. There were many long pauses between sucking bursts in protected infants when crying could have occurred, but simply did not. Moreover, there were a number of instances of crying in the midst of a sucking episode. Thus motor incompatibility as the genesis of sucking effectiveness is not supported. Secondly, differences can not be attributed to rate within a bout because for all infants intrabout rate was essentially 2.0/s. In this regard, analgesia can not be attributed to bout length per se because some protected infants had only 1 or 2 very protracted bouts, whereas others who had much shorter bouts, appeared to be equally protected as seen in the middle chart of Fig. 4.

Thirdly, analgesia can not be attributed to a sudden burst of sucking commencing with heel stick that prevented crying or that shifted the infant into a new state. Suck rates during heel stick were essentially stable relative to baseline. Indeed, rate diminished as often as it increased. Fourthly, the differences do not reflect one group of infants being asleep and the other awake. Our subjective impression at the time of data collection was that pacifier removal every 30-s was equally resisted by all infants. Fifthly, the diminished level of crying was paralleled by grimacing reduction (not shown for clarity reasons). In fact, grimacing patterns could almost be mapped onto those of crying. Thus, the hypothesis of motor incompatibility is difficult to reconcile

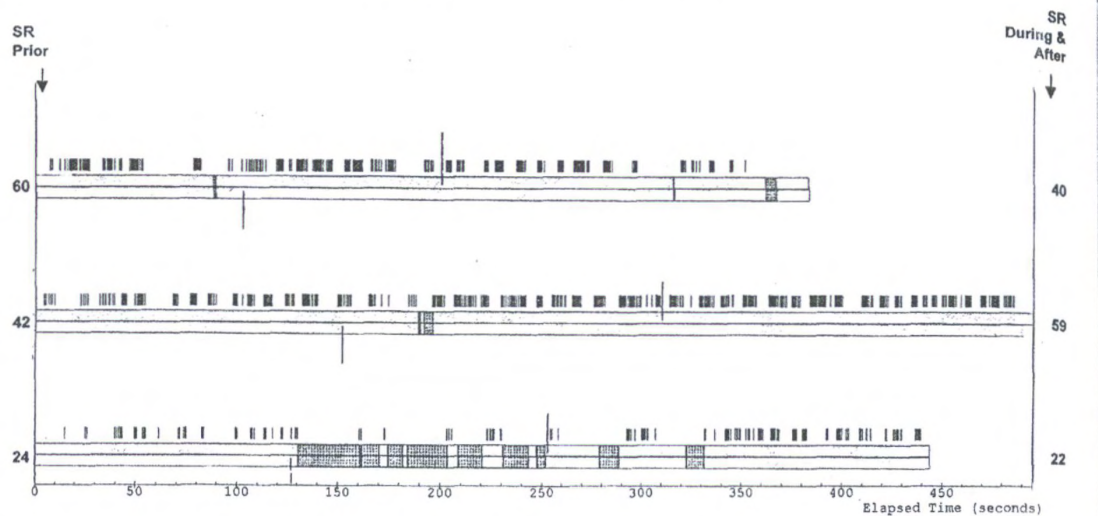


Fig. 4. Sucking and crying patterns over the course of the experiment for three infants representing fast, moderate and slow suck rates. Records begin with the onset of pacifier delivery (Treatment) and end with Recovery. The infant with the fastest rate is presented in the top chart. Crying is indicated by the gray and black shading where black is crying and gray, not crying. Sucks are indicated above the continuous bars. Suck rate calculated during delivery period is on the left-hand column and rate during heel stick and recovery is on the right. Vertical dashed lines represent collection onset and termination.

with lack of forehead and brow furrowing during suprathreshold levels of sucking. Rather, we suggest that either the afferent bombardment of sucking, the perception of oral motion, or the intensity of the sucking act, gained access to a central analgesia mechanism and this was manifest in the modest levels of crying and grimacing during and following heel lance. Finally the behavior of two infants in this sample who came to the experiment crying vigorously (Infants 17B and 42B in Fig. 3), reinforce the view that suck rate and not initial state determines reactivity to painful stimulation. Each infant, although crying when entering the study, was quieted when sucking the pacifier during the Treatment period. One sucked at a leisurely rate (17 sucks/min), the other at a somewhat higher rate (42 sucks/min). Their differences in crying and grimacing in response to heel stick were profound and entirely consistent with the findings regarding rate and analgesia from infants who were calm at study onset.

Mean heart rate changes during the entire lance procedure are presented numerically in Fig. 3 above the columns for each newborn. In general, heart rate appeared linked to crying and grimacing, but not always. For example, the exceptional infants are noteworthy because for them HR diminished in the face of painful stimulation and increased motor activity. Group data for HR change are presented in Fig. 5: according to paired *t*-tests mean heart rate increased substantially for infants in the water alone ($P = 0.002$) and water pacifier ($P = 0.005$) conditions from Treatment to heel lance (mean increases = 17 and 20 BPM, respectively), and then dropped gradually. Mean HR also increased marginally for infants in the sucrose pacifier

group ($P = 0.05$) with a mean difference of 7.4 BPM. HR changes for infants who received sucrose alone was not statistically reliable ($P = 0.142$) with a mean difference of 5.9 BPM. This accords with previously published data and provides a metric to help evaluate the effectiveness of the interventions in pain and stress reduction. HR differences were statistically reliable during the first 90 s of the recovery period between infants who received sucrose on a pacifier and infants in the two water conditions ($P = 0.04$ for water alone and $P = 0.03$ for water pacifier). The HR differences for infants who received sucrose, although suggestive, did

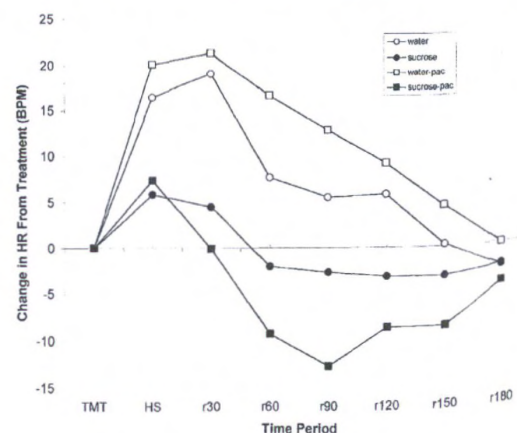


Fig. 5. Mean delta heart rate values for each treatment group. Change was calculated for each infant as the mean value for period minus the mean value during the Treatment period.

not attain statistical reliability during the Recovery period. What remains a general problem with the use of heart rate as an index of pain and stress is that the cause of change can not be ascribed with confidence. Increased heart rate may reflect perceived pain. It may also be indexing increased activity, crying in particular.

The relationship between suck rate and change in heart rate is presented in Fig. 6 for the ten infants who received a water-flavored pacifier (open symbols) and the ten whose pacifier was sucrose-flavored (filled symbols). The decrease is linear for infants in the water-pacifier group despite the marked increase in sucking activity. This is of interest when considering the relationship between sucking rate and energy conservation. In particular, heart rate decreased even though sucking activity was considerably elevated with its attendant increase in muscular output. The decrease, moreover, can not be attributed to differences in crying because there essentially was not any crying in infants whose suck rate exceeded 34/min. This is of potential clinical significance because it suggests possible differences in pain reactivity that were not reflected in crying or grimacing. The behavior of infants who sucked sweetened pacifiers will be addressed below.

3.2. Sucrose alone

Infants who received sucrose by syringe cried for 8% of blood collection time and grimaced for 19% of the time, as shown in Fig. 2. Seven of these ten newborns did not cry at all and five of the ten grimaced for less than 10% of the time

of blood collection. Grimacing may have been a more sensitive measure of pain during blood collection because five 'sucrose' infants, who did not cry, grimaced for at least 30% of the collection procedure. Tasting sucrose for the 2 min preceding heel lance muted the increased heart rate that characterized water controls during and following lancing, as shown in Fig. 5. The mean increase of 6 BPM is not statistically reliable and is extremely modest in comparison with the 20 BPM in water controls and infants who sucked a pacifier. Thus, sucrose taste engages antinociceptive mechanisms that persist well after sucrose delivery has terminated. As such sweet taste is a most potent behavioral inducer of analgesia for the substantial majority of newborn and premature infants who have been studied.

3.3. Pacifier-sucrose

Individual crying and grimacing outcomes for the ten newborns sucking a sweet pacifier are presented in Fig. 7. Only one infant cried to the heel stick, and an additional two frowned for about 10% of the time. With these exceptions none of the infants who received sucrose-pacifier treatment either cried or made grimacing expressions during the heel lance procedure or thereafter (not shown). The fact that the suck rate of 2 of these infants was well below 30/min, demonstrates that in circumstances of sweet orogustatory stimulation, suck rate does not have to exceed threshold in order for the combined treatment to be effective. The power of this phenomenon is appreciated from the fact that we were not able to tell when the heel lance was made in

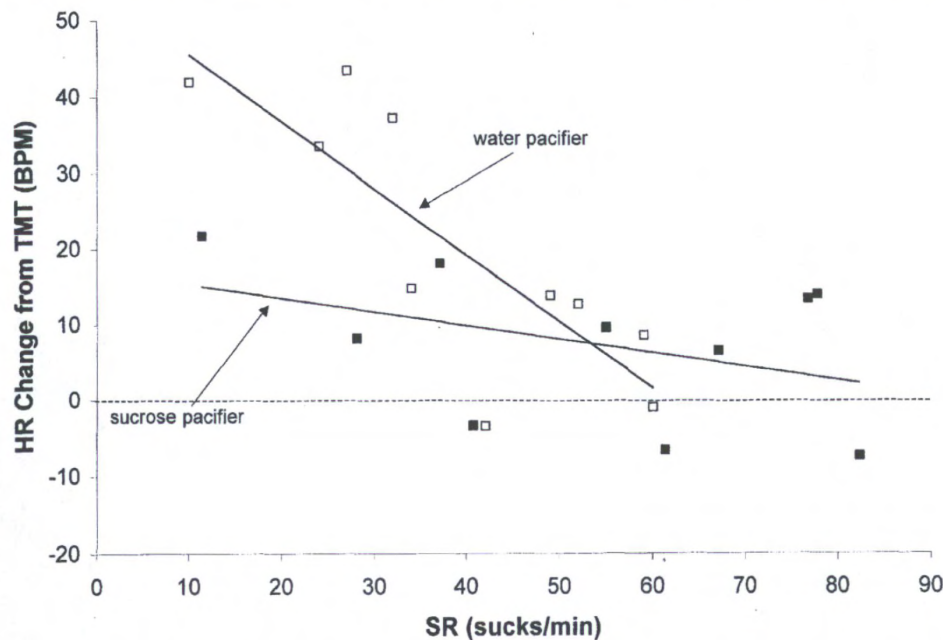


Fig. 6. Heart rate changes during heel lance in relation to suck rate is presented for water pacifier and sucrose pacifier groups.

seven of the ten infants tested and an additional four in preliminary studies. The differences between the sucrose pacifier and sucrose alone groups are not large. This reflects, we believe a basement phenomenon. Differences between groups would emerge during more severe stimulation, circumcision, for example, as reported by Blass and Hoffmeyer (1991) in which multisensory bombardment was more effective than single sensory afference for crying.

Heart rate outcome supports the conclusions that sucking a sweet pacifier actually blocked pain perception. Mean heart rate increase for sucrose alone and sucrose pacifier was 6-8 BPM during blood collection and declined very rapidly thereafter, actually reaching below baseline levels (Fig. 5). As shown in Fig. 6, the regression line relating change in HR to sucking rate is essentially flat across a wide range of sucking activity for infants who sucked a sucrose-flavored pacifier. This is of considerable interest from the perspective of energy conservation. It implies that sucrose taste, especially in combination with pacifier sucking, reduces heart rate and possibly energy loss independent of any change in motor activity.

It is worth commenting here on the exceptional infant presented in Fig. 7 because 'exceptional infants' have appeared in all of our previous studies. As in the present report, about 90% of the infants who we have studied have been markedly quieted by sucrose (Blass et al., 1989; Smith

et al., 1990; Blass and Smith, 1992). For the exceptional infants, there was no obvious effect of sucrose on crying or grimacing. It was not as if pain inhibition was muted, there was simply no evidence for pain relief according to our measures. We do not know the etiology of this failure. These infants, however, are members of a population that may prove to be of considerable interest for long-term studies on pain management, sensory physiology, and ingestive behavior during development.

Finally, there was excellent concordance between crying and grimacing incidence, as presented for all infants in this study in scatter-plot form (Fig. 8). Although a grimacing face often accompanied crying, it did not always. Moreover, grimacing occurred during times of silence in all infants who cried. Thus, grimacing linkage with crying reduction and minimal change in heart rate in infants who received sucrose alone, or on a pacifier, before and during blood collection, strongly suggests that grimacing reflects the underlying cause of pain perception and is not causally linked with tissue injury *per se*.

4. Discussion

The present findings are of theoretical and clinical interest. They have demonstrated the particular circumstances under which analgesia is caused by pacifier sucking in

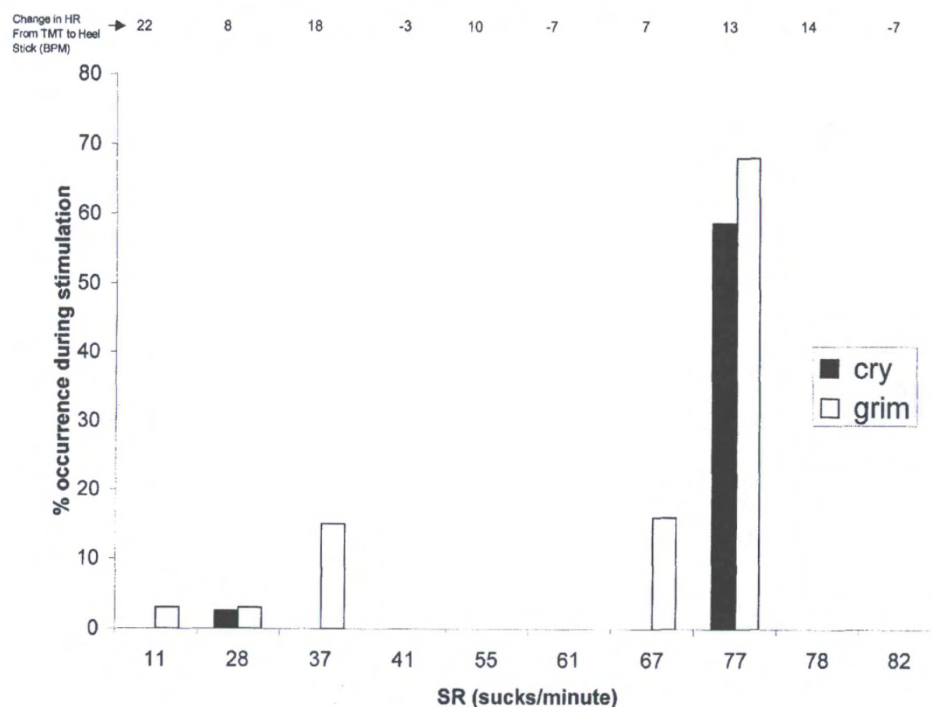


Fig. 7. Percentage of time crying and grimacing for individual infants in the sucrose-pacifier group presented in order of initial suck rate. Change in heart rate from Treatment to heel lance is indicated above the individual infants cry and grimace scores.

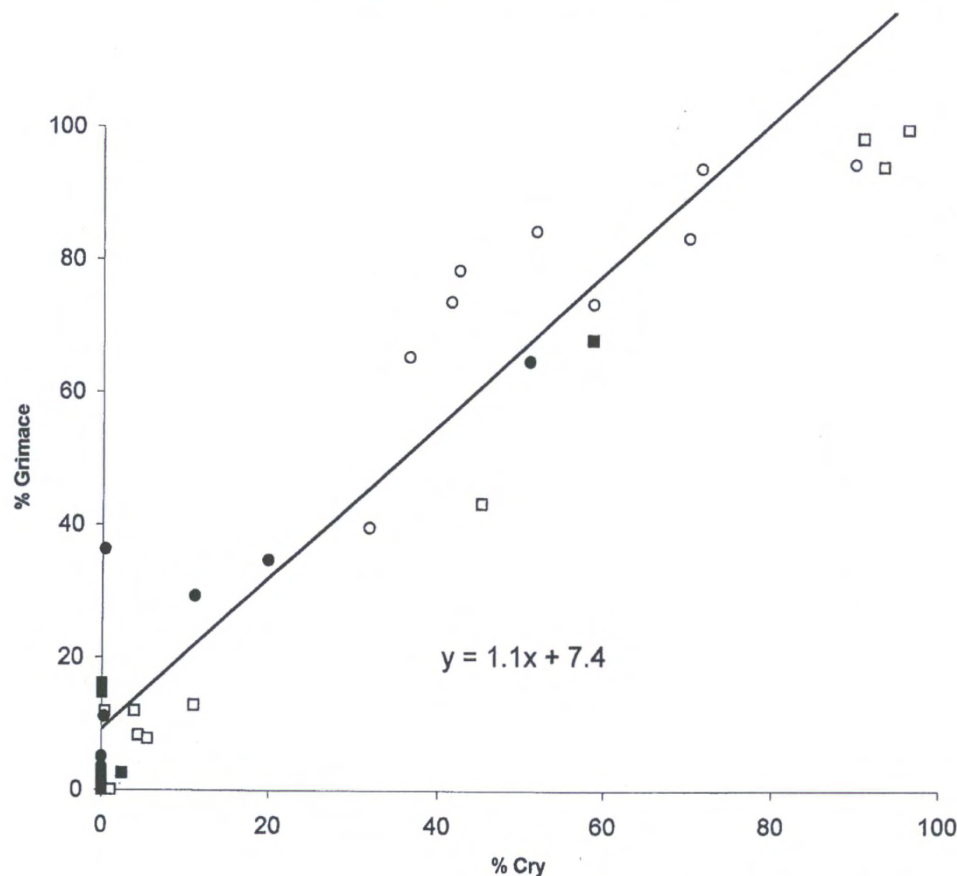


Fig. 8. Relationship between % grimacing and % crying for infants in all conditions.

term infants and provide direction towards identifying underlying mechanisms. They also reveal a synergism between orogustatory and orotactile mechanisms that had not been previously identified in human infants. Finally, the striking agreement between crying and grimacing incidence (Fig. 8) extends the range in which facial grimacing may be used clinically as an index of pain relief. This lends further weight to the argument advanced by Grunau and Craig (1987) that grimacing is an especially revealing measure of pain in populations such as premature infants for whom crying is infrequent, difficult, and metabolically expensive (Rao et al., 1997).

4.1. Possible artifacts

Perhaps the analgesia induced by sucking represents the putative incompatibility of sucking and pain expression. This can be rejected out of hand for grimacing which was operationally defined as brow furrowing and eye closure, the

indices found to be highly reliable by Craig et al. (1993). Likewise, incompatible motor responses can not account for the very modest increase in heart rate (circa 12 BPM) in infants sucking a pacifier at or above threshold rates.

We also find it unlikely that crying reduction was artifactual. Infants who responded to painful stimulation through crying and grimacing, did in fact suck the pacifier, some almost as much as the protected infants, and, like infants who had higher incidence of sucking, resisted our removing the pacifier every 30 s. Also, there were long intervals between sucking episodes of protected infants in which crying could have occurred during blood harvesting, but did not. The only distinguishing feature in our view between infants who were pain reactive, and those who were nonreactive while sucking a plain pacifier, was the greater number of sucking actions emitted by the later group. Thus, as in analgesia induced in rats in different phases of sucking, analgesia induced in human infants through sucking reflects central changes and should not be attributed to response competition.

4.2. Pacifier-induced analgesia

Although this report has not identified the mechanisms underlying SIA, it does provide interesting parallels with analgesia induced in rat infants and adults and in human adults through pleasurable, vigorous activity. According to Blass and Blom (1996), contact and suckling provide graded degrees of analgesia in 10-day-old rats against punctate thermal nociception. In particular whole-body contact with mother or siblings was more protective than isolation. NNS trebled heat escape latencies and rats were essentially analgesic during the full-body hyperextension response that is elicited during milk letdown. Unlike analgesia induced by milk flavor or sweet taste, none of the contact or suckling induced analgesias were blocked by naltrexone, even at the very high dose of 5 mg/kg.

The limited data that are available on underlying mechanisms in human infants are consistent with the rat findings. Infants born to women who were maintained on methadone during pregnancy were not quieted by sucrose but were by a pacifier during spontaneous crying (Blass and Ciaramitaro, 1994). These data are suggestive only because it is likely that other substances, both licit and illicit were used during pregnancy (Zuckerman et al., 1989; Frank et al., 1993). Finally, it is important to note that time parameters differ between suckling and gustatory induced analgesias. Protection conferred by NNS is essentially, for the moment, as its effects dissipate, generally within 30 s of suckling termination in both humans (Smith et al., 1990) and rats (Blass and Blom, 1996). In contrast, as shown here and in other studies (Blass and Shah, 1995), sucrose and milk (Blass, 1997a,b) induced analgesias and stress relief endure for at least minutes beyond substance infusion and this can not be attributed to lingering taste.

The function that is normally served by such potent antinociceptive mechanisms may be better conceptualized within the energetics of mother-infant interactions. Interactions that yield infant analgesia are the ones through which infants obtain milk. In both instances the infant's energy profile is improved. Firstly, calories and other commodities are derived from milk. Secondly, because of analgesia and stress reducing changes caused by sucking and taste, energy is conserved through crying reduction or avoidance, by reducing gross motor activity and by avoiding physiological adjustments that cope with stimuli that may no longer be perceived as painful. Sucrose taste, which stopped spontaneous crying in term and premature infants, reduced their energy expenditure by 13% (Rao et al., 1997). It is of interest in this regard that heart rate decrease was inversely and linearly related to suck rate when water was on the pacifier and was flat when sucrose was the tasted fluid. These findings hold considerable implications for understanding the relationship between sucking and energy conservation. We are currently directly evaluating the consequences of sucking on heat loss. Crying and grimacing in response to painful stimulation and its termination with sucking and sweet

taste may also be viewed as parts of a communication system that attracts and holds the caretaker's attention. Both communicative and energetic systems may function synergistically to favor selection of these multiply determined traits.

4.3. Sucrose

The behavior of infants who tasted sucrose accords well with previous reports on the analgesic effects of sweet taste in term and premature infants (Blass, 1996; Stevens et al., 1997; Blass et al., 1999; Barr et al., 1999). Five infants did not cry at all and six showed minimal facial grimacing during blood collection. The magnitude of sucrose induced analgesia in the current study reflects sucrose potency against a less traumatic procedure at the hands of the very experienced and skilled phlebotomist who performed 90% of the lances. From a clinical perspective, sweet taste as an analgesic agent in human term and premature newborns has now been verified many times with a variety of sweet tastes, including noncaloric substances (Stevens et al., 1997; Barr et al., 1998). Sweet tasting fluids are readily accepted by newborns and are easy to deliver. To our knowledge, no untoward effects have been reported in the literature or informally. That sweet taste is also analgesic in infant rats, and probably through the same mechanisms, speaks to the phylogenetic conservation of this multifaceted system.

Based on an extensive series of rat studies we conclude that orogustatory blockade was opioid based, presumably through the periaqueductal gray region. Although this has not been established directly, we have demonstrated that microliter intraventricular injections of morphine (Kehoe and Blass, 1986) or the milk derived opioid beta-casomorphine (Blass and Blom, 1996), confined in the ventricular circulation, cause analgesia. Moreover, analgesia caused by systemic administration of either morphine or beta-casomorphine was blocked by microgram injections of naloxone restricted to the ventricular space.

4.4. Sucrose-pacifier

The most potent behavioral analgesia that has been reported in both rat and human newborns is obtained when NNS is combined with tasting a sweet solution. With the exception of a single infant in the present study, analgesia appeared to be complete. Specifically, crying and grimacing were reduced from 50 and 70%, respectively, to essentially zero. There was a fleeting increase in heart rate at the beginning of blood collection that had normalized within 30 s following the end of the blood draw, indeed, heart rate actually fell below baseline levels. From the perspective of energetics, we would predict that sucking a sweet pacifier would cause the greatest reduction in energy loss in either mildly stressed or resting infants and that this

would be independent of suck rate. Studies to evaluate this hypothesis are currently underway.

Behavioral and immunohistological studies in rats provide direction to the locus of pain reduction in rats. Antinociception and pain blockades are likely to be occurring at the level of the dorsal horn of the spinal cord. In particular, rats that had received an injection of the inflammatory agent 'Complete Freund's Adjuvant' into a forepaw, were behaviorally hyperresponsive both to heat stimulation (hyperalgesia) and to touch (allodynia). The hyperalgesia and allodynia were reflected anatomically by dense immunoreactive staining labeled for the early gene *c-fos*, specifically in the third and fourth dorsal horn lamina at the cervical level of the spinal cord, indicating extensive early gene activity in these pain pathways (Ren et al., 1997).

Suckling the nonlactating nipples of an anesthetized female while receiving oral infusions of sucrose, completely reversed the allodynia and hyperalgesia in such rats and essentially eliminated the increased early-gene activity (Ren et al., 1997). This suggests that pain was actually blocked at its source at the level of the spinal cord, as opposed to reduced once it had reached the brain. This was presumably achieved via the descending ventrolateral fasciculus from periaqueductal gray to the point of entry of pain afferents in the dorsal horn. The fact that neither response latencies to hindpaw stimulation, nor *c-fos* levels in the sacral cord were affected by NNS-sucrose interventions, because the descending dorsolateral fasciculus had not yet reached its target, supports the spinal interpretation of pain alleviation (Fitzgerald et al., 1988, 1989; Fitzgerald, 1995). These findings are also consistent with a report by Yi and Barr (1995) who found that analgesia induced by intracerebroventricular injections of morphine was blocked by severing the descending ventrolateral fasciculus in 10-day-old rats.

Obviously these findings can not be replicated in human infants. We are encouraged, however, to speculate about the blockade of painful afferent bombardment with the combination of sucrose and pacifier sucking on three grounds. Firstly, all of the studies that others and we have conducted to evaluate similarities and differences between rat and human newborns in the properties of suckling-induced analgesia have demonstrated identical characteristics between the two species.

Secondly, the basic organization of neurological and neuropharmacological (Fitzgerald and Bond, 1991; Fitzgerald, 1995) mechanisms underlying pain and its relief in the spinal cord and base of the brain in rats and humans has been conserved. Thirdly, seven of the ten infants who received sucrose on a pacifier before, during, and after heel lance, either cried not grimaced at any time during or following the procedure. The absence of crying and grimacing is striking, especially given how readily they occurred in control infants during heel lance. Based on these findings, we speculate that the pain pathways normally activated by local

tissue trauma were not activated so that pain sensation did not reach the brain.

4.5. Clinical implications

Although the view is widely held that pacifier sucking is calming, only very few studies have evaluated its antinociceptive effects. Group reductions of crying, for example, have been modest, as in the present study (Fig. 2). Previous designs did not lend themselves to evaluating, in very small detail, the relationship between some aspect of sucking prior to a painful intervention on pain perception during the procedure. Suck rate prior to a procedure, some covariant, or correlative was the determining factor in this study. This is of clinical importance because pacifiers are routinely kept in the newborn's bassinet and seating and maintaining a pacifier in the infant's mouth is not at all difficult and can be smoothly incorporated into the standard procedure preparation routine.

Sweetened pacifiers provide an even more potent and easily implemented treatment against local pain in newborns that can be applied in complete safety, and with no apparent risks of side effects. Sweet solutions can be readily given to infants on a pacifier in advance of and during necessary treatments or evaluations that may be moderately painful. This combination may also be profitably implemented for more painful and enduring procedures such as circumcision. Blass and Hoffmeyer (1991) reported an almost 70% reduction in crying in infants who had been circumcised while sucking a sweetened pacifier. A sweetened pacifier should be administered in combination with standard treatments such as EMLA cream or dorsal penile nerve block that use peripheral pathways and do not have a central component. In a recent study, sucking a sweet pacifier caused substantial reductions in HR increase during circumcision (Herschel et al., 1998). Suckling sweetened pacifiers has the added advantage of combating nonsurgical stress involved in the procedure, most notably restriction to the circumcision board. The recent report by Shah et al. (1998) is relevant in this regard because paracetamol delivered 60–90 min prior to blood collection did not diminish crying or grimacing, so that at least this pharmacological intervention is not an option in the treatment of newborn pain. Behaviorally induced analgesias either through taste, flavor, suckling, or contact offer very promising, effective, and safe, noninvasive and nonpharmacological treatments.

Sucking a sweet pacifier may induce a preventive analgesia, promoting a less stressful recovery from painful procedures. The effects of this intervention may be enduring because Taddio et al. (1995) have demonstrated that males circumcised at birth cried more at the time of their 4-month inoculation than did uncircumcised males or females. Reducing the pain content of the traumatic event may also reduce longer-term reactivity to painful stimulation. In this regard sucking a sweet pacifier may supplement more aggressive means of controlling infant pain as with opioid administra-

tion following surgery (Anand et al., 1987; Anand and Hickey, 1992).

4.6. Exceptional infants

It has repeatedly been our experience that circa 10% of the infants whom we have studied during the past decade have not been at all responsive to sweet taste. This poses interesting clinical problems in identifying these infants prior to interventions so that alternative means can be selected. These infants may help identify potential deficits in coping with pain and unusual patterns of ingestive behavior.

4.7. Facial grimacing

Finally, these studies have demonstrated the utility of facial grimacing as a metric of pain reduction. Although this research does not address the larger issue of whether grimacing is better conceptualized as a communicative signal of pain perception or simply as a response *per se* to pain (Craig et al., 1984), it is fair to say that we have demonstrated that grimacing reflects the perception of a painful event. It is decidedly not tissue damage *per se* that elicits the grimace. Because of the linkage of crying and grimacing in healthy term infants as a reflection of pain (Fig. 8), grimacing may prove especially useful in assessing the effectiveness of pain interventions in premature or other at-risk infants, who frequently are subject to repeated heel sticks and other painful procedures and who can not cry in any sustained fashion.

In summary, we have identified the circumstances under which sucking a pacifier induces analgesia, and the effects of combining nonnutritive suckling with sucrose taste. This combination of opioid and nonopioid mediated processes yields a remarkable analgesia in both rat and human infants.

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CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e), the undersigned certifies that Patent Owners Exhibits 2023-2066 are being served on February 8, 2024, by filing Patent Owners Exhibits 2023-2066 through the Patent Trial and Appeal Case Tracking System (P-TACTS) and by electronic mail as directed by Petitioner upon the following counsel of record for Petitioners:

David B. Conrad
Lance E. Wyatt
Sarika Patel
Casey Kraning
Fish and Richardson P.C.
To: IPR53953-0001IP1@fr.com

/Mark E Ungerman/
Mark E. Ungerman
(Reg. No. 32,070)