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REGULAR ARTICLE

The flexion withdrawal reflex increases in premature infants at 22–26 weeks of gestation due to changes in spinal cord excitability

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ABSTRACT

Aim: Our aim was to study the development of the cutaneous flexion withdrawal reflex among premature infants admitted to the neonatal intensive care unit of the Children's Hospital, University of Cologne, in 2013.

Methodology: This longitudinal cohort study explored the development of spinal cord excitability of 19 premature infants born at 22–26 weeks of gestation. We performed five investigations per subject and studied changes in the reflex threshold with increasing postnatal age at different behavioural states. The premature infants were stimulated with von Frey filaments on the plantar surface of the foot near the first metatarsophalangeal joint during the first 3 days of life and at postnatal ages of 10–14 days, 21–28 days, 49–59 days and a corrected gestational age of 37–40 weeks.

Results: The mean gestational age of the premature infants included in the study was 24 weeks. Premature infants with a gestational age of less than 26 weeks presented a flexion withdrawal reflex with a low threshold (0.5–2.85 milli-Newton) in the first 72 hours of life.

Conclusion: The flexion withdrawal reflex among premature infants born at less than 26 weeks showed a continuous threshold increase with increasing postnatal age, reflecting changes in spinal cord excitability.

INTRODUCTION

Premature infants with a gestational age of less than 26 weeks can experience pain and present motor responses, depending on the development of nociceptive circuits (1–4). On the other hand, non-noxious motor responses, associated with the constantly changing spinal excitability of these infants, also present a unique field that can be explored (1). The functional development of the dorsal horn in neonates is characterised by a gradual shift from excitation to inhibition (1). The initial input from the A fibres that stimulate the excitatory interneurons is later balanced by the input from the brainstem and the C fibres that stimulate the inhibitory interneurons. Infants have stronger nociceptive as well as excitatory reactions compared to adults (1).

A promising approach for quantifying spinal excitability in infants is the measurement of cutaneous sensitivity using the cutaneous flexion withdrawal reflex, which is a painless noninvasive tool (5). Studies among term infants have displayed a threshold increase during the first year of life, while in preterm infants a significant threshold increase has been associated with increasing postnatal age (5–11). In addition, a unilateral decrease of the reflex threshold has been associated with injuries of the examined limb prior to the reflex examination (12). The reflex threshold has been reported to be significantly lower among lightly sleeping term infants, in comparison with quietly awake subjects throughout the first year of life (5). However, these studies have some limitations regarding the generalisability and

Abbreviations
IQR, Interquartile range; IVH, Intraventricular haemorrhage; mN, milli-Newton; NICU, Neonatal intensive care unit; SD, Standard deviation.

Key notes
- This longitudinal cohort study examined the development of the cutaneous flexion withdrawal reflex in premature infants born at 22–26 weeks and admitted to a neonatal intensive care unit.
- The 19 infants were stimulated on the foot at various ages up to a corrected gestational age of 37–40 weeks.
- We found that the flexion withdrawal reflex showed a continuous threshold increase with increasing postnatal age, reflecting changes in spinal cord excitability.

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transferability of their results, since infants with a minimum gestational age of 25 weeks have not been included in the study population very often.

Our hypothesis was that infants with a gestational age of 22–26 weeks would present an increase of the threshold of the flexion withdrawal reflex with increasing postnatal age. We examined the reflex as a motor response to increasing mechanical force stimulation, performed using calibrated von Frey filaments on the plantar surface of the foot.

METHODS

We conducted a longitudinal cohort study, enroling premature infants with gestational ages of 22–26 weeks during the first 3 days of life. Every infant was investigated five times during their neonatal intensive care unit (NICU) stay. All infants were in a stable cardiorespiratory state, with fraction of inspired oxygen <0.3 after endotracheal surfactant application at the time of the initial investigation performed during the first 3 days of life. The subsequent investigations were carried out at the postnatal ages of 10–14 days, 21–28 days, 49–59 days and finally at a corrected gestational age of 37–40 weeks that corresponded to a postnatal age of 11–18 weeks depending on the gestational age. The investigations were only performed among subjects that received noninvasive respiratory support. The study was carried out at the Children's Hospital of the University of Cologne in 2013.

We excluded infants with congenital anomalies, severe metabolic diseases or serious neurological diseases – including intraventricular haemorrhage (IVH) grade 2 or higher before the recruitment (13) – cystic periventricular leukomalacia or those that had received sedation, muscle relaxation, opioid or other analgesic drugs within 24 hours of the first investigation. Subjects who received muscle relaxation, sedation or analgesics within 24 hours of any investigation, or who had received bilateral heel pricks within 12 hours of any investigation, were excluded from that particular investigation, but were included in further investigations. If subjects had received a unilateral heel prick within 12 hours of an investigation, the investigation was conducted on the contralateral limb. Subjects that developed IVH were included in the study, and the incident was recorded in the case report form.

The Ethics Committee of the Medical Faculty of the University of Cologne approved this study. Informed consent from parents or guardians was obtained prior to recruitment. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. No infant was subjected to pain because of the study.

There was no skin contact with the subjects prior to the investigations. The flexion withdrawal reflex was examined on one limb at a time. We used calibrated von Frey filaments made of nylon with logarithmically arranged monofilaments VF1 Optihair (MARSTOCKnervtest, Hessen, Germany) to provide the stimulus. The force was applied at a range of 0.25–362 milli-Newton (mN). One trained investigator performed the investigations directly after infant feeding, with the subjects lying supine in the incubator or bed. The stimulations were applied perpendicularly on the plantar surface of the foot near the first metatarsophalangeal joint (5). Each investigation was performed once, starting with the same minimal force of 0.25 mN to ensure that the reflex threshold was not reached at the first stimulation (14). A brisk flexion of the limb away from the stimulus signalled that the threshold had been reached. The minimum force leading to withdrawal of the limb was regarded as the reflex threshold.

To explore relevant threshold variations among preterm infants at different behavioural states, we compared the reflex thresholds of subjects that were in quietly awake and in lightly asleep behavioural state before the investigations (15,16). The last investigation was only performed on quietly awake subjects.

A total of 19 subjects, three female and 16 male, were investigated longitudinally. We extracted perinatal data and results from routine cranial ultrasound examinations during the NICU stay from the patient records and documented these using pseudonyms. Statistical analyses we carried out using STATA 14 (StataCorp, Texas, USA). Because of the small cohort size and the explorative nature of the study, we limited the analyses to nonparametric tests. We used the Friedman test to compare the paired data of the five investigations and Pearson's chi-square test to compare reflex thresholds between awake and asleep subjects.

RESULTS

The subjects had a mean gestational age of 24 weeks with a standard deviation (SD) of 6.93 and a median of 23 weeks and 6 days with an interquartile range (IQR) of 22 weeks and 3 days to 26 weeks. The mean birth weight was 627 g (SD 164.5 g), and median was 590 g (IQR 370-985 g). No infant suffered from ultrasound-evident IVH in the first 3 days of life. Between the first two periods – the first 3 days and 10-14 days – 1–10 infants suffered from different grades of IVH: five subjects presented with IVH grade 1, two had IVH grade 2, and three had IVH grade 3. There were no cases of IVH after the tenth day of life. None of the infants was diagnosed with cystic periventricular leukomalacia until discharge (17).

We completed all investigations within the first 3 days of life. The infants also received at least two additional investigations. One infant was under sedation, and three infants were in transport during the timeframe of the investigations at 10–14 days, 21–28 days and a corrected age of 37–40 weeks. The threshold values are summarised in Table 1. The individual reflex thresholds and the behavioural state of the subjects are displayed in Table 2.

We conducted a nonparametric Friedman test of differences between the repeated measures to see if the reflex threshold increased across time with increasing age. The analysis revealed a statistically significant chi-square value

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Table 1 Longitudinal documentation of the withdrawal reflex thresholds.

Investigation	Threshold among all subjects	Threshold among awake subjects	Threshold among asleep subjects
1–3 days			
median	0.71 (0.50–2.85)	0.855 (0.50–2.85)	0.71 (0.50–2.85)
mean	1.13 (0.76)	1.159 (0.74)	1.11 (0.83)
N	19	10	9
10–14 days			
median	2.00 (0.71–11.3)	3.00 (0.71–11.3)	1.40 (1.40–2.85)
mean	3.39 (3.06)	4.35 (3.71)	2.02 (0.76)
N	17	10	7
21–28 days			
median	2.85 (1.40–128)	4.275 (1.40–128)	2.425 (1.40–4.00)
mean	10.51 (29.42)	16.75 (39.20)	2.71 (1.16)
N	18	10	8
49–59 days			
median	8.0 (1.40–32.0)	8.0 (1.40–32.0)	4.275 (2.00–16.0)
mean	11.38 (10.08)	13.76 (11.02)	6.23 (5.28)
N	19	13	6
11 to 18 weeks (gest. age 37–40 w)			
median	19.30 (2.85–64.0)	19.30 (2.85–64.0)	
mean	25.33 (18.20)	25.33 (18.20)	-
N	18	18	

Significance of Friedman Test: χ^2 = 27.5527 (p = 0.000).
Postnatal age 11 to 18 weeks and gestational age 37 to 40 weeks.
Results presented as following: median (IQR), mean (SD) in mN. Statistical analysis of the reflex threshold with increasing postnatal age (Friedman test).

Table 2 Investigated withdrawal reflex threshold in mN. Behavioural state is marked as: quietly awake (a), and lightly asleep (s)

Code	Gest. Age	Threshold 1st invest. (state)	Threshold 2nd invest. (state)	Threshold 3rd invest. (state)	Threshold 4th invest. (state)	Threshold 5th invest. (state)	IVH (4th–10th day of life)
01	23w3d	0.50 (s)	–	2.00 (a)	2.85 (a)	22.6 (a)	None
02	26w	1.40 (a)	5.70 (a)	11.3 (a)	32.0 (a)	16.0 (a)	None
03	24w5d	2.00 (a)	8.00 (a)	128 (a)	32.0 (a)	45.3 (a)	None
04	25w1d	2.00 (s)	11.30 (a)	5.70 (a)	22.6 (a)	8.00 (a)	None
05	23w	0.71 (a)	0.71 (a)	1.40 (s)	2.00 (s)	5.70 (a)	None
06	25w6d	0.71 (s)	–	5.70 (a)	22.6 (a)	2.85 (a)	III°both sides
07	23w6d	2.85 (s)	1.40 (s)	2.85 (a)	2.85 (s)	45.3 (a)	None
08	23w3d	0.71 (a)	1.40 (s)	–	8.00 (a)	45.3 (a)	None
09	24w	0.50 (s)	1.40 (a)	2.00 (a)	16.0 (s)	–	None
10	23w3d	1.00 (a)	2.85 (s)	4.00 (s)	8.00 (a)	45.3 (a)	III°both sides
11	23w2d	2.85 (a)	8.00 (a)	2.85 (a)	8.00 (a)	16.0 (a)	I°both sides
12	23w6d	1.00 (a)	1.40 (s)	1.40 (a)	2.85 (s)	16.0 (a)	III°both sides
13	23w5d	0.71 (a)	1.40 (s)	2.85 (s)	5.70 (s)	22.6 (a)	I°right
14	22w3d	0.50 (s)	2.00 (a)	2.00 (s)	2.85 (a)	5.70 (a)	I°both sides
15	23w2d	0.71 (a)	1.00 (a)	1.40 (s)	1.40 (a)	22.6 (a)	None
16	23w1d	1.00 (s)	2.85 (s)	4.00 (s)	8.00 (a)	45.3 (a)	I°bds.
17	24w	1.40 (s)	4.00 (a)	5.70 (a)	8.00 (a)	11.3 (a)	II°bds
18	24w	0.50 (s)	1.40 (a)	2.00 (s)	22.6 (a)	64.0 (a)	II°left, I°right
19	25w3d	0.50 (a)	2.85 (s)	4.00 (s)	8.00 (s)	16.0 (a)	I°right

of 27.5527, p = 0.000) (Table 1). There was a strong positive correlation between postnatal age and the reflex threshold. The threshold increase is graphically demonstrated in Figures 1 and 2.

We analysed threshold differences based on the infants' behavioural state in each one of the first four investigations using Pearson's chi-square test. Although Figure 2 seems to imply a lower reflex threshold among lightly sleeping

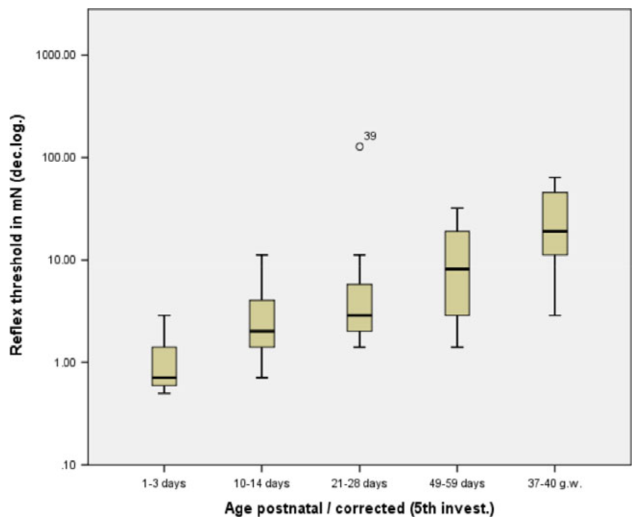

Figure 1 Threshold of the flexion withdrawal reflex at all five investigations. Box plots represent median value and IQR (max, min.) 39 and 63 are outliers (more than 1.5 IQR but less than 3 IQR from the box plot). The scale of the *y*-axis is decadic logarithmic.

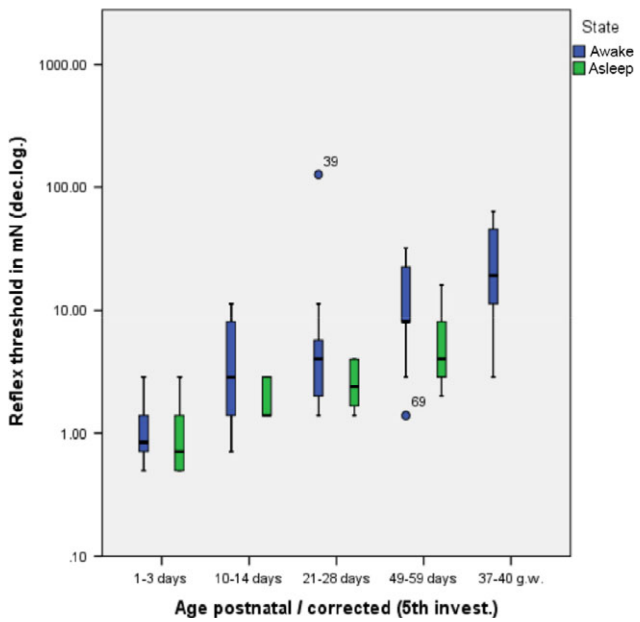

Figure 2 Threshold of the flexion withdrawal reflex at all five investigations, differentiated for infants being quietly awake or in light sleep. Box plots represent median value and IQR (max, min.) 39 and 63 are outliers (more than 1.5 IQR but less than 3 IQR from the box plot). The scale of the *y*-axis is decadic logarithmic.

infants at first glance, the analysis did not reveal significant differences.

DISCUSSION

To our best knowledge, this was the first longitudinal study of flexion withdrawal reflex among infants with a gestational age of 22–26 weeks. The studied infants presented the reflex as a motor response to increasing mechanical force stimulation using von Frey Filaments during the first 3 days of life, with a median threshold value of 0.71 mN (IQR 0.50 – 2.85 mN). The study of the reflex on the third day of life among term newborns revealed a much higher threshold with a median threshold value of 12 mN (IQR 8 – 20 mN) (5). We also found a threshold increase with increasing postnatal age. Similar increases in the threshold have also been reported among term infants during their first year of life (5).

Abdulkader et al. (8) reported a median threshold of 3 mN among extremely premature infants with a mean gestational age of 31 weeks (25–35 weeks). The timing of this investigation corresponded to a period that was chronologically placed between the investigations at 21–28 days (median 2.85 mN) and 49–29 days (mean 8 mN) of our study. These findings lend support to the theory of the maturation process of spinal excitability with a postnatal increase of the reflex threshold. This has often been reported in the literature with regard to premature infants with a higher gestational age and among term infants (5,10,11).

The subjects investigated in our study at a corrected age of 37–40 weeks, the group examined by Kühne et al. during the first 3 days of life (5), and the nonsensitised full-term group explored by Abdulkader et al. (8) were all of a similar corrected age. The respective median threshold values were 19.3 mN for our group, 12 mN for the Kühne et al. group (5) and 5 mN for the Abdulkader group (8)**.** This revealed a difference in the threshold values of the preterm infants at a corrected age 37–40 weeks in comparison with the term infants. These differences in stimulus perception between healthy term and preterm infants may be explained by hypersensitivity through repetitive noxious stimulation of the latter (18). Differences in the skin cornification processes (19) in utero and in a NICU setting may explain the finding. Differences in the haptic perception of premature infants during their NICU stay may be relevant for the changes in the reflex threshold, particularly in comparison with term infants (20,21).

Kühne et al. (5) reported higher threshold values at 3 days of age compared to 4 weeks. High concentration of placental neuroinhibitors – such as neuroactive steroids like progesterone and 3α-hydroxyl metabolites – and enzymes such as 5α-reductase and prostaglandin D2 in the umbilical cord blood may have contributed to this result. Such neuroactive steroids have been detected in human foetal plasma from 20 weeks of gestation and in human foetal skin, central nervous system, kidneys, small intestines, liver and lungs from 17 weeks of gestation (22). It is unclear to what extent a relevant effect may be displayed among premature infants with a gestational age of less than 26 weeks. We could not reproduce these findings in our study, probably because the two study populations were not comparable (5).

Considering the synaptic developmental changes in the dorsal horn, a lower reflex threshold could be considered a defensive reflex of an organism that is still unable to present a coordinated pain response (1). The withdrawal reflex in sleeping term infants showed a significantly lower threshold compared to quietly awake term infants throughout the first year of life (5,15), which was probably associated with this protective' character of the reflex during sleep (15). Our statistical analysis did not reveal significant differences between awake and asleep subjects, possibly due to the small cohort size or because of differences in skin cornification and maturity (19).

A reflex threshold decrease has been reported among term and preterm infants when investigating a limb that has already received one or more heel pricks. Multiple heel pricks may lead to hyperalgesia (8,12). Differences in the reflex threshold have been reported in association with the anatomical side of the stimulation or the stimulation of the contralateral limb (10,11). To rule out any influence from these factors, we formulated appropriate inclusion and exclusion criteria for this study. We only performed investigations on the limb that had not received any heel prick in the preceding 12 hours. This study partially shared the study population with another study on noxious responses among premature infants (23).

Surface electromyography recordings using self-adhesive electrodes have been used to measure the reflex threshold among premature infants of gestational ages of 30–41 weeks (7,24). In this study, however, we did not use any additional skin electrodes because of the immaturity of the premature infants' skin. We also chose to use a more conventional definition of the flexion reflex threshold (5).

There are fewer births at this gestational age, and our cohort size was small. This posed a major limitation for our study. The study was performed in a single NICU and the transport of premature infants to other departments during their NICU stay complicated the recruitment and data collection processes. We performed statistical analyses appropriate to the small sample size.

Another limitation was that a single investigator conducted the investigations. On the other hand, a single trained investigator does rule out problems of inter-rater variability with regard to the definition of the reflex threshold. We worked with a strict study protocol, which increased reproducibility.

We did not evaluate differences in the muscle tone of the patients that could lead to varying answers even after a standardised stimulation. We did not assess infants' motor performance in the supine position, to check for a possible activation of the tonic labyrinth reflex.

We would like to highlight the difficulties in identifying the subjects' behavioural state during the first few days of life. Presentation of fine general movements (16) or spontaneous eye movements was regarded as signs of a quietly awake state. Future research should consider the subjects' behavioural state, potentially using electrophysiological methods.

CONCLUSION

Premature infants displayed a flexion withdrawal reflex after stimulation with von Frey filaments as early as a gestational age of 22 weeks and 3 days. The reflex appeared with a very low threshold during the first 3 days of life in comparison with term newborns. The threshold increased continuously up to a corrected age of 37–40 gestational weeks, reflecting the changing spinal cord excitability in human development (1,24,25). Although we did not identify significant threshold differences between awake and asleep infants, further studies should consider the infants' behavioural state. The small cohort size, due to fewer births at this gestational age, was a major limitation of our study.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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