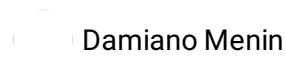# 🌐 Studying yawning behavior in preterm neonates before and after feeding

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This protocol can be used to study yawning behavior in preterm neonates hospitalized in NICUs.

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Exclusion criteria

1 Exclusion criteria: Gestational Age ≥ 37 weeks; Congenital anomalies, heart or metabolic disorders, fetal infections, clear teratogenic factors, Apgar at five minutes < 6 and grade III or IV hemorrhages.

Procedure

2 Neonates for whom consent was obtained from parents should be observed two times, respectively before and after scheduled feedings, while they are lying supine in a cot. All of the video-recordings should take place in the afternoon, while the neonates are not receiving any stimulation through routine nursing or medical care, and should last around 30 minutes. All of

the neonates should be video-recorded both before and after feeding.

Behavioral Coding

3   Frame by frame behavioral analysis of video-recordings should performed by two independent coders expert in behavioral micro-analysis (with the secondary coder examining 30% of the videorecordings, n = 36), using ELAN, a professional software for the creation and management of complex annotations on video and audio (Max Planck Institute for Psycholinguistics, The Language Archive, Nijmegen, The Netherlands; http://tla.mpi.nl/tools/tla-tools/elan/).

4   **Yawn coding**

Yawns should be identified holistically according to the *System for Coding Perinatal Behavior* (*SCPB*), which is based in part on selected facial Action Units (AUs) from the anatomically based *Baby FACS: Facial Action Coding System for Infants and Young Children* and previous studies in the literature. For reliability assessment purposes, once an event is identified as a yawn based on this description, the onset should be scored at the first frame where mouth opening is visible.

Yawning is defined in the SCPB as a stereotyped behavior characterized by a slow mouth opening with deep inspiration, followed by a brief apnea and a short expiration and mouth closing, typically accompanied by limb stretching. The expansion of the pharynx can quadruple its diameter, while the larynx opens up with maximal abduction of the vocal cords. One of the characteristic features of yawning is its timing, consisting in a progressive acceleration, followed by an abrupt deceleration in the intensity of the facial muscle Action Units (AUs) involved, designated by numeric codes and verbal labels. Yawning usually emerges from a relaxed face, initially involving mouth opening (AUs 25, 26, 27) and eyes closing (AU 43E), followed by upper eyelid drooping (AU 43A-D), flattened tongue on the bottom of the mouth (AU 75) and usually swallowing (AU 80). During the plateau brow knitting (AU 3), brow knotting (AU 4), nose wrinkling (AU 9), lateral lip stretching (AU 20), nostril dilatation (AU 38) and head tilting back (AU 53) also typically occur.

5   **Quiet sleep coding**

Quiet sleep (regular sleep or non-REM sleep, QS) periods should be scored when the following conditions are met for at least three consecutive minutes: primarily abdominal respiration which is regular in rhythm and constant in amplitude, fully closed eyelids and no movements except for occasional startles, sudden jerks or rhythmic mouthing.

Data analysis

6   Inter-rater reliability between the primary and secondary coder should be calculated using Cohen's Kappa, with .70 being the conventional threshold for acceptable reliability. In particular, reliability can be assessed for the occurrence of yawning by adopting a one-second threshold, while a 30 seconds threshold can be used for calculating Kappa for quiet sleep scores.

7   Hierarchical Poisson regressions with participant ID as random intercept can be used to compare yawning occurrences before and after feeding, as well as to test other associations with other variables. The natural logarithm of the observation time should be used as offset variable.