Cortical route for facelike pattern processing in human newborns

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Humans are endowed with an exceptional ability for detecting faces, a competence that, in adults, is supported by a set of face-specific cortical patches. Human newborns, already shortly after birth, preferentially orient to faces, even when they are presented in the form of highly schematic geometrical patterns vs. perceptually equivalent nonfacelike stimuli. The neural substrates underlying this early preference are still largely unexplored. Is the adult face-specific cortical circuit already active at birth, or does its specialization develop slowly as a function of experience and/or maturation? We measured EEG responses in 1- to 4-day-old awake, attentive human newborns to schematic facelike patterns and nonfacelike control stimuli, visually presented with slow oscillatory “peekaboo” dynamics (0.8 Hz) in a frequency-tagging design. Despite the limited duration of newborns’ attention, reliable frequency-tagged responses could be estimated for each stimulus from the peak of the EEG power spectrum at the stimulation frequency. Upright facelike stimuli elicited a significantly stronger frequency-tagged response than inverted facelike controls in a large set of electrodes. Source reconstruction of the underlying cortical activity revealed the recruitment of a partially right-lateralized network comprising lateral occipitotemporal and medial parietal areas overlapping with the adult face-processing circuit. This result suggests that the cortical route specialized in face processing is already functional at birth.

What are the neural bases of this early bias for faces in the human baby brain? Is there a universally shared neural system that newborns deploy when processing faces vs. other kinds of stimuli? The earliest evidence available to date for an early neural response to faces comes from EEG and fMRI/PET studies in infants already 2 to 4 mo old. The EEG studies compared the event-related potentials (ERPs) evoked by canonically oriented faces vs. inverted faces (17, 18) or vs. noise images with equivalent low-level visual properties (19), or contrasted the response to novel vs. familiar faces (20); in all cases, faces elicit a higher amplitude of the N290 and/or P400 ERP waves at occipitotemporal electrodes. A recent study in 4- to 6-mo-old infants using a novel EEG frequency-tagging paradigm (see more below) alternative to ERPs, confirms these results by showing a clear response to faces (compared with objects/scenes) in right lateral occipitotemporal electrodes (21). Although none of these studies attempted to reconstruct the anatomical sources of the EEG effects, their results are broadly compatible with the occipitotemporal neural generators of specific face-processing ERP signals seen in adults (22). The only fMRI investigation on face-specific cortical responses in infants (23) confirms that the large-scale organization of face-selective regions in the adult is already present at 4 to 6 mo after birth (fusiform gyrus, lateral occipital cortex, superior temporal sulcus, and medial prefrontal cortex; see also ref. 24), although with a lower-category selectivity (such face-selective regions respond to objects much more in infants than in adults). These results are suggestive of an early cortical protoarchitecture that preferentially engages when stimulated with faces. However, given the fast development of the visual system during the first 3 mo (25), it remains an open question whether (and to what extent) the same occipitotemporal circuit

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involved in face processing in infants and adults is already active at birth—when newborns’ experience with faces is still extremely limited—or whether such specialization emerges only later as a function of experience and/or maturation.

Here, we aim to bridge this gap by investigating the electro-physiological correlates of processing facelike stimuli in awake, attentive human newborns of less than 96 h after birth. We presented newborns with schematic and canonically oriented facelike stimuli (upright faces) and, as controls, with an inverted version of the same stimuli (inverted faces) (8, 9). As an additional control, we also presented “scrambled” faces organized in a nonfacelike, top-heavy fashion (more elements in the upper part than in the lower part of the oval) to investigate a previously proposed hypothesis that the preference for upright faces at birth may be mainly determined by a general preference for stimuli in which geometrical organization is top heavy vs. bottom heavy (26).

To comply with the extremely short duration of focused attention in newborns (27), we took advantage of a frequency-tagging paradigm—a design that “tags” the neural populations coding for a given stimulus by presenting that stimulus periodically at a specific (tag) temporal frequency and by measuring the neural response in the form of a sharp peak in the EEG power spectrum at the same frequency (28). Since both the EEG ongoing activity and EEG artifacts are broad band in frequency, the stimulus-related response in the frequency domain is easily discriminated from the stimulus-unrelated activity with relatively light artifact rejection, yielding a much higher signal-to-noise ratio (SNR) than the one obtained with ERPs. Oscillating visual stimulation based on the same principle has been widely used in the pioneer work on low-level visual function in newborns (e.g., refs. 29 and 30).

We used a high-density (125 electrodes) EEG system with a cap specifically designed for newborns (Electrical Geodesic, Inc.) to record EEG activity in 1- to 4-old healthy human newborns while presenting them with streams of schematic upright, inverted, and scrambled faces (Fig. 1) presented periodically at a frequency of 0.8 Hz. Newborns’ stimulus-related brain responses were quantified from the peaks of the EEG power spectrum at the frequency of stimulus presentation.

Recent empirical and simulation studies showed that the newborn-scalp EEG topography is much less spatially smeared compared with that of the adult [the spatial decay of focal transients in newborn EEG signals is approximately three times steeper than the corresponding decay in adult EEG recordings (31)]. This property is generally attributed to the significantly thinner skull bones of the newborn. These studies suggest that by using a high spatial sampling of the scalp EEG and a realistic newborn head model and conductivity values (more than 1 order of magnitude higher than the adult ones) (31, 32), we could compute a reliable source reconstruction of the newborn’s EEG (33, 34). With these indications, taking advantage of high-density EEG recordings, we estimated the cortical generators of the scalp-level effects with a source localization model based on newborn’s realistic anatomical structure and electrical properties (34).

**Results**

Visual stimuli (upright, inverted, and scrambled geometric representations of faces, see Fig. 1) were presented dynamically with sinusoidal contrast modulation (0 to 100%) in blocks of 50 s (or until the subject stopped fixating) at a rate of 0.8 Hz (1 cycle = 1.25 s), overlapped onto a weakly contrasted dynamic white-noise background to minimize after-image effects. Data from the 10 subjects completing the protocol for all conditions were epoched on the basis of fixation intervals. After artifact rejection, the duration of clean EEG data per condition was, on average, 36.4 s (upright, 35.6 ± 17.6 s; inverted, 33.7 ± 13.7 s; scrambled, 39.9 ± 21.0 s), with no statistical difference among the three conditions (F(2,18) = 0.28, P = 0.68).

**All Stimuli Elicit a Frequency-Tagged EEG Response.** We first tested whether with such short data intervals we could reliably measure a significant oscillatory response at the frequency of stimulation. Given the steep 1/f-like profile of the power spectrum in the low-frequency range of the stimulation frequency in newborns (35), we estimated the stimulus-unrelated “background” power at the tag frequency by a power-law fit of the power spectrum at neighboring frequency bins (±0.3 Hz). We then investigated the presence of a frequency-tagged response (FTR) by testing whether (and for which) electrodes the power at the tag frequency was significantly higher than the estimated background power. Statistical testing for this and for all of the following analyses was performed with a permutation-based nonparametric algorithm that tests the effects on the whole set of electrodes with no prior region-of-interest selection; the issue of multiple comparison is overcome by directly assessing the statistical significance on spatial clusters of channels (ref. 36; see Materials and Methods).

The results showed that at the tag frequency, the oscillating stimuli (all conditions merged) elicited a significantly higher power than the estimated background power in a large set of posterior electrodes (Pcorr < 0.003) and in a smaller frontal cluster (Pcorr < 0.022) (Fig. 24). Visualization of the power spectrum in the posterior cluster shows that, as expected, this effect is due to a high peak of power at the tag frequency emerging from a 1/f-like profile at neighboring frequency bins (Fig. 2B).

When conditions were considered separately, all stimuli elicited a significant peak at the tag frequency in a posterior cluster (upright, Pcorr < 0.004; inverted, Pcorr < 0.024; scrambled, Pcorr < 0.020), while only upright stimuli gave rise to an additional peak in a frontal cluster (Pcorr < 0.013) (SI Appendix, Fig. S1).

**The Electrophysiological Signature of Facelike Pattern Processing.** Legitimated by the previous analyses, we quantified the FTR to each kind of stimulus as the ratio between the amplitude of the power spectrum at the tag frequency and the background power at the same frequency, estimated by the power-law fit as above.

With that measure in hand, we moved to the direct investigation of the main focus of our research: characterizing the electrophysiological signature of processing facelike patterns by statistically comparing the FTR to facelike patterns, first to inverted and then to scrambled patterns. Compared with inverted faces, faces elicited a stronger FTR (Fig. 3B) in a wide posterior, slightly right-lateralized cluster (Pcorr < 0.003) (hereafter indicated as the main cluster) and in an anterior right-lateralized cluster (a weaker but significant effect; Pcorr < 0.049) (Fig. 3.4). Remarkably, the effect in the main cluster was very robust (effect size: d = 2.49), consistently present in every single newborn (Fig. 4B) and independent from looking-time differences (correlation coefficient between the difference of the response to upright vs. inverted faces and the corresponding difference in looking times, R = 0.09, P = 0.8). In the following, we denote facelike pattern response as...
Fig. 2. FTR, all conditions merged. (A) Statistical map (one-tailed t test, corrected) of the difference between the power spectrum at the tag frequency (0.8 Hz) and the background power at the same frequency, estimated by a power-law fit of the power spectrum from the six neighboring frequency bins (±0.3 Hz). Electrodes belonging to a statistically significant cluster are marked with a black dot. Two clusters emerge: a posterior one (P corr < 0.003) and a frontal one (P corr < 0.022). (B) Power spectrum averaged over electrodes belonging to the posterior cluster (with P < 0.01) (black line) ± SEM (gray shadow) across subjects: although the overall frequency profile is well described by a power law (dashed dark-gray line, fitted in the interval 0.5 to 1.1 Hz), a peak neatly emerges at the tag frequency.

the difference between the response to upright faces and the response to inverted faces.

The Effect of Age on the Facelike Pattern Response. To test the impact of age/exposure to faces on the facelike pattern response, we performed a correlation between age (in hours after birth) and the average facelike pattern response in the main cluster. Results showed a significant negative correlation (R = 0.71, P < 0.02) (Fig. 3D).

Cortical Sources of the Response to Facelike Patterns. To estimate the cortical generators of the facelike pattern response identified at the sensor level, we used an anatomical model morphed to newborns’ anatomy (34) to compute a detailed model of the infant head and cortical folds. We then used this forward model to reconstruct a plausible distribution of the cortical origins of our scalp recordings (see Materials and Methods).

The areas associated with the facelike pattern response at the source level (Fig. 3C) comprise a network that appears mostly lateralized to the right hemisphere and includes areas both along the occipitotemporal and the occipitoparietal stream: Along the ventral stream, activity emerges in bilateral occipital regions extending laterally to the right inferior occipital/posterior fusiform gyrus, superiorly toward the right posterior superior temporal sulcus, and anteriorly toward the right anterior temporal lobe. A strong activation was also seen in medial posterior regions, including the right precuneus and cuneus. Finally, some activation was observed in the right superior frontal gyrus.

To confirm that the spatial resolution of the source-reconstructed data is sufficient to support the anatomical segregation described in Fig. 3C, we tested the spatial spreading of the source reconstruction of surrogate, biologically plausible EEG data generated by simulated oscillatory cortical sources centered in key areas (SI Appendix, Fig. S2A; see SI Appendix, SI Materials and Methods for details). SI Appendix, Fig. S2B shows that the spatial spreading of all the simulated sources was limited to a contiguous neighborhood, supporting the reliability of the anatomical distinction between the activations shown in Fig. 3C.

Response to Scrambled Faces Is Intermediate. Lastly, we investigated the response to scrambled faces to test the hypothesis that they may yield the same pattern of response to upright faces due to their top-heavy configuration. However, contrary to the comparison of upright vs. inverted faces, the FTR to scrambled faces was not higher as compared with inverted faces (no significant clusters, P > 0.05 for all uncorrected single-channel t tests), nor was it significantly different from upright faces (P corr > 0.07). To further explore the nature of this intermediate response, we computed the FTR to scrambled faces in the main cluster associated with the facelike pattern response: Although the average power spectrum is more similar to inverted faces than to upright faces (Fig. 4A), the response to scrambled faces is very variable across subjects (Fig. 4B), being closer to upright faces in some subjects (4/10) and closer to inverted faces in others (6/10).

Discussion

The Mature Cortical Face Network Is Present Early in Newborns. In this study, we used a frequency-tagging paradigm combined with high-density EEG to show that human newborns display a face-selective neural activation revealed by a higher response to face-like geometric patterns than to tightly controlled visual stimuli.

The estimated cortical sources of such response (Fig. 3C) extend along the occipitotemporal pathway in areas including those found in most adult fMRI studies on face processing with intracranial EEG recordings using frequency tagging in adults (37); and with fMRI/PET in infants as young as 2 to 6 mo old (23, 24): inferior occipital gyrus (consistent with the location of the occipital face area), fusiform gyrus (even if more posterior than the location of the fusiform face area), posterior superior temporal sulcus, and anterior ventral temporal lobe [see SI Appendix, Fig. S3 for a comparison with the “core system” of face processing in adults (4)]. These results suggest that at least a subset of the cortical face-processing network is already laid down and functional in newborns.

In addition to this occipitotemporal right-lateralized activity, we observe a medial activation centered in the precuneus—a region associated with memory and attention and thus potentially reflecting the higher involvement of these processes in elaborating upright faces vs. inverted faces. Interestingly, face-processing
studies in adults using fMRI report a higher activity in the pre-
cuneus for familiar vs. unfamiliar faces (38), which may tenta-
atively suggest that the high precuneus activation we observe here
could reflect an early-developed familiarity to facelike patterns
compared with nonfacelike ones.

Response to Facelike Patterns Does Not Increase with Age. Although
newborns spend most of their time sleeping, when they are
awake, the visual stimuli that they are more frequently presented
with are upright faces (16). A study by Farroni et al. (39) indi-
cated that the intensity of the near-infrared spectroscopy signal
recorded in right occipitotemporal channels while newborns
were viewing dynamic faces indeed increased with age in infants
from 24 to 120 h after birth. This was taken as evidence that the
cortical face-specific response requires frequent exposure to
to develop. However, the reported correlation is difficult to
interpret, as the activation to faces was not directly contrasted
with that of a control condition, leaving open the possibility that
its increase with age reflected a general maturation of the visual
system. Indeed, our results are incompatible with the idea that
the face-specific cortical response increases as a function of ex-
posure to faces, because the correlation between age and the
facelike pattern response is significantly negative (Fig. 3D). In
a speculative attempt to account for this surprising finding, we
remark that we used highly simplified facelike geometrical pat-
tterns that, for newborns, act as key or supernormal stimuli (in
ethical terms; see ref. 40), the sensitivity to which was pre-
viously shown to rapidly decrease already within the first month
of life (8). One possible explanation is that while such key stimuli
are optimally fit for the immature visual system of the newborn
in the very first hours of life, experience with real-world complex
and variable faces may refine the facelike circuitry such that it
rapidly gets more attuned to the real-world features and gradu-
ally loses sensitivity to artificial facelike geometrical patterns.
This fascinating but speculative possibility deserves further
testing with a larger sample of newborns; for example, by com-
paring the developmental trajectory of the cortical response to
facelike patterns and real-world faces.

The Role of Cortical and Subcortical Structures. An influential the-
ory proposes that newborn preferences for facelike stimuli may be
mainly generated by a subcortical route involving the superior
colliculus, amygdala, and pulvinar (41). This theory, however,
mainly relies on the assumption that the cortical visual route
compared with the subcortical one is very immature in newborns
and on indirect behavioral evidence that the face-preference
phenomenon occurs only through the temporal visual route
(42). One alternative possibility is that the processing of visual
information proceeds in multiple waves of activation that involve
both subcortical and cortical structures (43), a hypothesis sup-
ported by the dense connectivity of the structures of the sub-
cortical visual route with multiple areas of the cortex (43) and
by recent evidence from resting-state fMRI studies that, by term age,
the newborn cortex has reached a highly organized functional
architecture (44) and thalamocortical connectivity (45), similar in
many aspects to those of the adult.

Our study cannot provide evidence for or against an in-
volvement of the subcortical route in face processing. In fact,
because subcortical structures generate extremely weak electrical
fields due to their closed-field geometry and because they are far
from the scalp, they hardly produce measurable signals at the scalp
level (46). Therefore, we believe that an impact of subcortical
activity on our EEG results is unlikely and, thus, did not include
subcortical areas in our source reconstruction analysis.

However, our source reconstruction results support the hy-
pothesis of a recruitment of a specific set of cortical structures in
facelike processing at birth. Since this network overlaps with the
adult face-processing circuit, we further speculate that one or
more of these cortical areas might be already sensitive enough to
facelike stimuli to generate the orientation preference to facelike
patterns observed in newborns (7, 8). It is worth noting that this
cortical recruitment is fully compatible with an intrinsic subcorti-
cal route of the visual input (42), alternative to the relatively
immature lateral geniculate nucleus/primary visual cortex pathway,
because the pulvinar and amygdala are densely connected with (and
massively influenced by) multiple cortical areas (43).

Sensitivity of the EEG Frequency-Tagging Paradigm over Behavioral
Measures. Interestingly, while the early behavioral preference for
upright facelike patterns compared with inverted ones is sys-
tematically observed in newborns by using preferential looking
paradigms (whereby two different stimuli are concurrently shown
on the screen; e.g., ref. 9), with single central presentations similar
to the one used in the current stimulation paradigm, a behavioral
preference for faces over nonfacelike controls is typically not de-
tected until 2 mo of age (47). However, EEG responses to our
centrally presented single stimuli did indicate a strong FTR dif-
ference across conditions, suggesting that, in this case, direct brain
measures can be more sensitive compared to behavioral measures.

Faces or Top-Heavy Configurations? Another result of the current
experiment is that the face-deprivation response to scrambled faces
compared with upright faces and inverted faces. The fact that
scrambled faces did not elicit a stronger FTR than inverted faces
does not support the hypothesis that face preference reflects a
preference for top-heavy configurations (26). In other words,
the presence of a top-heavy configuration alone is not sufficient to
systematically elicit a facelike neuronal response. However, even
if, on average, upright faces elicit a higher FTR compared with
scrambled faces, the high intersubject variability suggests that top-
heavy stimuli may sometimes be categorized as a face.

The Role of Experience in Early Face Processing. Recent studies show
that after prolonged face deprivation from birth, monkeys do not
exhibit any looking preference for faces (15, 48) and, unlike
normally reared monkeys, they do not develop any fMRI-
measured face-selective domains (48). Our findings are not in
contradiction with these results for two main reasons. First, both
Arcaro et al.’s (48) and Sugita’s (15) studies tested face selec-
tivity in monkeys reared in face deprivation (interacting only with
masked human caregivers) for at least the first 3 mo after birth.
We believe that face selectivity at the test time is likely to be
strongly influenced by the experience of face deprivation during
this long and critical period of development, whereas it is poorly
informative about face selectivity at birth. Interestingly, both the
fMRI responses and looking times in ref. 48 indicate that during
the face-deprivation period, monkeys developed a high selec-
tivity for hands and body parts, the most relevant stimuli in the
interaction with their caregivers. One potential speculation is that this environment-specific social learning might have caused the “unlearning” of their early predisposition to encode faces (if there was such a predisposition), a form of high functional plasticity that systematically occurs in animals with sensory deprivation (49); notably, face selectivity is not irreversibly lost but is recovered upon late exposure to faces, even after 2 y of face deprivation (15). On the other hand, contrary to face-deprived monkeys, our newborn subjects did have a normal, albeit very limited, exposure to human faces, and this might have been sufficient to boost potentially predetermined face-specific cortical responses.

Second, fMRI studies in normally reared monkeys failed to reveal significant “face patches” before 6 mo of age (50). However, because monkeys show a clear face-biased preference much earlier on, the presence of a cortical patch that is entirely and selectively dedicated to faces is not necessary for encoding (and preferring) faces. What, then, is the neural underpinning of face preference in monkeys? There are two possibilities that are coherent with the absence of face patches: Face-responsive neurons might already exist at birth but (i) they might be less spatially segregated from the nonface-responding neurons compared with the adult’s cortical organization, or (ii) their degree of face selectivity might be weaker than that observed in adults’ face neurons [as is observed in 4- to 6-mo-old human infants (23)]. Both scenarios are likely to provide null results with the mass-univariate subtraction-based fMRI data analyses performed in refs. 48 and 50, whereas face-specific responses might be revealed using multivariate pattern analysis methods, which, unfortunately, were not performed in the aforementioned studies. Our study, which does not rely on the assumption of the amount of spatial segregation of face-selective neurons and which integrates a very powerful low-level visual control (inverted faces), clearly shows that it is possible to detect face-specific neural responses as early as 1 to 3 d from birth.

Future Directions. Newborns spend most of their time sleeping, and during the rare periods in which they are calm and awake, their visual attention typically lasts no more than 3 to 5 min (27). Here, we show that the frequency-tagging paradigm provides a valid tool for measuring in newborns high SNR brain responses to multiple stimulus-specific conditions with very short stimulus presentation (around 40 s per condition), confirming results obtained with older infants (21, 51) and opening the way to investigate the neural substrates of other core perceptual/cognitive functions in this very special population. The high statistical significance of the face-like bias effect, reflected in its presence for each single subject, suggests that our experimental protocol, even in its shorter version limited to the presentation of upright and inverted faces, might be used as a biomarker to test the sensitivity to face-like patterns in populations at risk (like autism spectrum disorder) as a complement to behavioral tests on visual social predispositions (52).

Materials and Methods

The study was approved by the local ethical committee for clinical research (Comitato Etico per le Sperimentazioni Cliniche, Azienda Provinciale Servizi Sanitari, Province of Trento, Italy) and was performed in the maternity ward of Rovereto Hospital Santa Maria del Carmine. Parents were informed about the content and goal of the study and gave their written informed consent. The data that support the findings of this study are available from the corresponding authors upon reasonable request by individual scholars.

Subjects. Ten newborns (six males; mean age 60 ± 22 h, range 15 to 96 h) were included. All were healthy (APGAR(1 min) ≥ 8, APGAR(5 min) = 10 for all subjects), born full term (gestation age, 39.7 ± 1.5 wk), and of normal birthweight (average weight, 3.41 ± 0.28 kg). Forty-four additional newborns participated but were excluded either because they did not complete the study (criteria: attend all three stimulus conditions for at least 20 s each) or due to inattentiveness (18), falling asleep (18), or crying (8) or because their data contained more than 10 EEG artifacts (mainly due to movements or high electrode impedance) (5).

Stimuli. See SI Appendix, SI Materials and Methods for details. In brief, visual stimuli (Fig. 1, Top) consisted of a white head-shaped form containing three black squares, and differed only in the spatial configuration of the three squares to form the three stimuli (upright face, inverted face, and scrambled face). Stimuli were presented dynamically with sinusoidal contrast modulation (0 to 100%) at a rate of 0.8 Hz, oscillated onto a weakly contrasted dynamic background (Fig. 1, Bottom) consisting of a flickering white-noise image. We used sinusoidal contrast modulation instead of squared on-off dynamics, both to minimize nonlinear effects in the brain frequency response (28) and to make the stimulation more pleasant to the babies (21). The slow presentation rate (0.8 Hz) was chosen to ensure that newborns fully perceived the stimulus at each cycle of the periodic, peekaboo-like presentation.

Experimental Protocol. Newborns were tested in a calm, dimly illuminated space in the maternity ward, seated on the lap of a trained researcher in front of an LCD screen (60 × 33.8 cm; distance from eyes to screen: about 30 cm) while wearing an EEG cap (see SI Appendix, SI Materials and Methods for details about the EEG system). Video recording from a hidden camera on the top of the screen ensured on-line monitoring of the infant. The newborn’s parents, when present, were off the sight of the infant (separated by a curtain), and instructed to keep silent during the recordings. Each trial started with a distractor (a gray spiral looming toward the center of the screen on a reddish background). As soon as the newborn started to fixate the center of the screen, stimulation started with 1 s of flickering of one of the three triplet stimuli, followed by a blank screen. The triplet was presented again, up to three times. Each triplet was centered off-line by a black square of the same size, as indicated by the experienced researcher (E.D.G.) who reviewed the video recordings blindly with respect to the experimental conditions. Newborns had an average fixation time of 43.4 s per condition. There was no statistical difference among fixation intervals in the three conditions [F(2, 18) = 0.24, P = 0.74].

EEG Data Analysis. Data analysis was performed with EEGLAB (53), FieldTrip (54), Brainstorm (55), and custom-made software based on MATLAB R2016b. EEG data were preprocessed for artifacts and segmented in blocks corresponding to fixation intervals (see SI Appendix, SI Materials and Methods for details).

Frequency-Tagging Analysis. To obtain a high-frequency resolution of the power spectrum with one bin centered on the stimulation frequency (0.8 Hz), epoch length was set to exactly eight stimulation cycles (10 s), resulting in a frequency resolution of 0.1 Hz. EEG data from each block were segmented in single-epoch power spectrum: \( P(f) = |F(f)|^2 \) where \( F(f) \) is the frequency-tagging effect, two-sided for the differences between conditions. EEG data were preprocessed for artifacts and segmented in blocks corresponding to fixation intervals (see SI Appendix, SI Materials and Methods for details). The power spectrum was calculated from these Fourier coefficients as the average over epochs of the single-channel power spectrum: \( P(f) = \frac{1}{N} \sum_{n=1}^{N} |F(f + nT)|^2 \). In the Fourier transform, the FTR at the tag frequency (0.8 Hz) was calculated as the ratio between the power spectrum at the tagged frequency and the value at 0.8 Hz of the power-law region of the power spectrum estimated from the six neighboring frequency bins (0.3 Hz), where the power-law fit was computed by fitting a line to the log-magnitude of the power at the six neighboring frequency bins (MATLAB function Polyfit). It is worth noting that, due to the steep 1/f-like power-law of the power spectrum in newborns in the low-frequency interval analyzed here (0.5 to 1.1 Hz) (35), the popular method to estimate the background power spectrum at the tag frequency by simply averaging over neighboring frequency bins (56) overestimates the background power (and therefore underestimates the FTR) because the power spectrum is much steeper for lower-frequency compared with higher-frequency bins around the tag frequency.

Statistical Analysis. The frequency-tagging effect was evaluated by comparing the logarithm of the power at the tag frequency with the logarithm of the background power estimated by the power-law fit described above. Differences between conditions were evaluated by comparing the logarithm of the relative FTRs. We tested the statistical significance of these effects with the nonparametric cluster-based test (36) implemented in FieldTrip (54). This method allows statistical testing with no need of a priori selection of regions of interest because it controls for multiple comparisons by clustering neighboring channel pairs that exhibit statistically significant effects. First, we tested at each channel point, dependent-samples t statistics; threshold, \( P = 0.05 \); one-sided for the frequency-tagging effect, two-sided for the differences between conditions) and using a permutation test to evaluate the statistical significance.
the at cluster level (Monte Carlo method, 2,000 permutations for each test). The P value of each statistically significant cluster is indicated as $P_{	ext{corr}}$ to mark that it is corrected for multiple comparisons. The effect size is estimated with a post hoc analysis on the statistically significant cluster by computing Cohen’s d as the ratio between the mean and the SD (across subjects) of the effect averaged over all of the electrodes of the cluster.

Source Reconstruction. See SI Appendix, SI Materials and Methods for details about the source reconstruction model. Source-level FTRs were estimated as follows: (i) For each subject, source-level time series were reconstructed from the segmented EEG data on the 8,014 sources obtained from the MNE reconstruction in Brainstorm; (ii) log(FTR) was estimated at the source level by using the same frequency-tagging analysis used at the sensor level; and (iii) the resulting source signal was spatially smoothed (10 mm).

For each contrast of interest, a paired t test was run at each source location, and the corresponding significant clusters ($P < 0.05$ uncorrected) are reported (et al., 2005). Source-level FTRs were estimated as important for brain responses to upright and inverted faces and cars. Dev Neurosci 38:272–280.


