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William P. Fifer  
*Columbia University*

Dana L. Byrd  
*University of Florida*

Michelle Kaku  
*Columbia University*

Inge-Marie Eigsti  
*University of Connecticut - Storrs*

Joseph R. Isler  
*Columbia University*

*See next page for additional authors*

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**Authors**

William P. Fifer, Dana L. Byrd, Michelle Kaku, Inge-Marie Eigsti, Joseph R. Isler, Jillian Grose-Fifer, Amanda R. Tarullo, and Peter D. Balsam

# Newborn infants learn during sleep

William P. Fifer<sup>a,b,c,1</sup>, Dana L. Byrd<sup>d</sup>, Michelle Kaku<sup>a</sup>, Inge-Marie Eigsti<sup>e</sup>, Joseph R. Isler<sup>b</sup>, Jillian Grose-Fifer<sup>f</sup>, Amanda R. Tarullo<sup>a</sup>, and Peter D. Balsam<sup>a,g</sup>

<sup>a</sup>Department of Psychiatry, Columbia University, New York, NY, 10032; <sup>b</sup>Department of Pediatrics, Columbia University, New York, NY 10032; <sup>c</sup>New York State Psychiatric Institute, New York, NY 10032; <sup>d</sup>Department of Psychology, University of Florida, Gainesville, FL 32611; <sup>e</sup>Department of Psychology, University of Connecticut, Storrs, CT 06269; <sup>f</sup>Department of Psychology, John Jay College of Criminal Justice, City University of New York, New York, NY 10019; and <sup>g</sup>Department of Psychology, Barnard College, Columbia University, New York, NY 10027

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**Newborn infants must rapidly adjust their physiology and behavior to the specific demands of the novel postnatal environment. This adaptation depends, at least in part, on the infant's ability to learn from experiences. We report here that infants exhibit learning even while asleep. Bioelectrical activity from face and scalp electrodes was recorded from neonates during an eye movement conditioning procedure in which a tone was followed by a puff of air to the eye. Sleeping newborns rapidly learned the predictive relationship between the tone and the puff. Additionally, in the latter part of training, these infants exhibited a frontally maximum positive EEG slow wave possibly reflecting memory updating. As newborns spend most of their time sleeping, the ability to learn about external stimuli in the postnatal environment during non-awake states may be crucial for rapid adaptation and infant survival. Furthermore, because eyelid conditioning reflects functional cerebellar circuitry, this method potentially offers a unique approach for early identification of infants at risk for a range of developmental disorders including autism and dyslexia.**

EEG | eyelid conditioning | neonate

During the first days of life, awake infants are capable of learning associations between oral motor patterns and altered milk flow (1) and can learn to alter sucking to produce a variety of reinforcers, including milk (2), their mother's voice (3, 4), or a sweet-tasting solution (5). Cross-sensory associative learning also has been demonstrated in awake neonates using paired auditory and visual stimuli (6, 7). Furthermore, awake newborns show Pavlovian conditioning to tactile (8) and taste stimuli (9, 10), as well as eyelid conditioning to paired auditory and tactile stimuli (11). These early adaptations to the postnatal environment have been well documented in awake infants, but as newborns spend the vast majority of their time asleep, the need and capacity to learn may not be confined to states of wakefulness.

Even while asleep, neonates are able to process external information actively. Scalp recordings of brain activity in sleeping neonates have demonstrated their capacity to differentiate between two sounds (12–14), indicating that infants are forming representations of specific stimuli and distinguishing between those stimuli during sleep. We report here that sleeping neonates not only process information about individual events, but also learn about relationships between them.

To investigate whether neonates can learn during sleep, we attempted to condition an eye movement response in 1- to 2-day-old infants while they slept. All infants were fed immediately before testing to increase the likelihood they would sleep through the entire procedure. Sleep status was confirmed using behavioral observations in conjunction with heart rate variability patterns, respiratory regularity, and video scoring of the infants' faces. Infants were videotaped while exposed to tones and puffs of air directed at the eyelid. In the experimental group, tones were a reliable signal that a puff of air was likely to be presented. In the control group, tones and puffs were presented at random times. In addition to video scored observational measures derived from previous studies with older awake infants (15, 16), electroencephalographic and muscle activity was also recorded

from a network of 124 scalp and face electrodes. Responses associated with both neural processing and eye movements to the tone and air puff were recorded and analyzed over the course of a single training session.

## Results

In the experimental group, participants averaged a 4-fold increase in the likelihood of the conditioned eye movement response (EMR) by the end of training, whereas the likelihood of responding did not change in the control group (Fig. 1*B*). Statistical analyses were conducted on data aggregated into five blocks of 40 trials. A two-way analysis of variance showed a significant difference between groups,  $F(1, 28) = 38.17, P < 0.05$ , and a significant group- (experimental vs. control) by-block interaction,  $F(4, 112) = 8.41, P < 0.05$ . Separate ANOVAs for the two groups showed a significant increase in eyelid responses over trials in the experimental group,  $F(4, 100) = 42.14, P < 0.05$ , but not for the controls. The group average onset latency of eye movement responses to the tone on tone-alone trials decreased from 1,030 ms in the first block of the session to 870 ms in the final block, becoming significantly closer to the expected puff onset at 900 ms,  $t(25) = 5.40, P < 0.005$ . By the end of training, 24 of the 26 babies in the experimental group had, at a minimum, doubled the likelihood of making an EMR. In contrast, there was no change in the probability of an EMR in the control group (Fig. 1*B*). Because  $\approx 92\%$  of the subjects in the experimental group conditioned, the chance of having 4 of 4 subjects that fail to condition (as in the control group) is  $< 0.00005$ . Hence, 4 subjects provide an adequate control for this behavioral paradigm. For the total sample, EMR was observed on  $> 90\%$  of puff-alone trials across all blocks, demonstrating that infants consistently were responsive to the unconditioned stimulus. There was no change in rate of unconditioned responding from the first to the second half of the session,  $t(23) = 1.09$  (not significant).

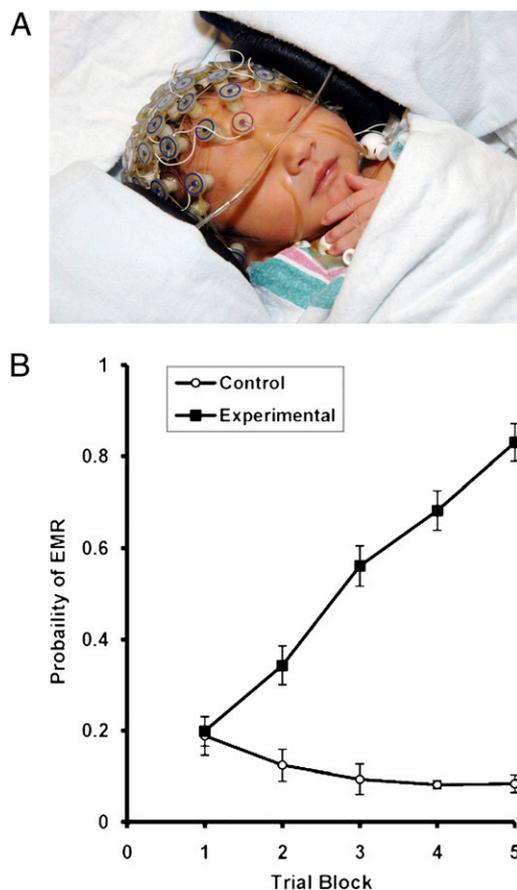
Because the auditory evoked potential is relatively small in comparison with the ongoing EEG, at least 50 trials were required to extract a clear sensory evoked potential using averaging. Ten infants in the experimental group, but only 1 in the control group, had sufficient artifact-free EEG responses required for event-related potential (ERP) analysis. Consequently, statistical analysis was possible only in the experimental group. In this subset, separate averages were constructed for each infant for the first and the second half of the conditioning trials. Consistent with previous studies of newborns (17–19), these babies had clearly visible auditory evoked potentials (AEPs) with a positive peak at  $\approx 300$  ms (Fig. 2). The AEP was maximal in amplitude (3.5  $\mu$ V) over the central areas. Visual inspection of

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<sup>1</sup>To whom correspondence should be addressed. E-mail: wpf1@columbia.edu.

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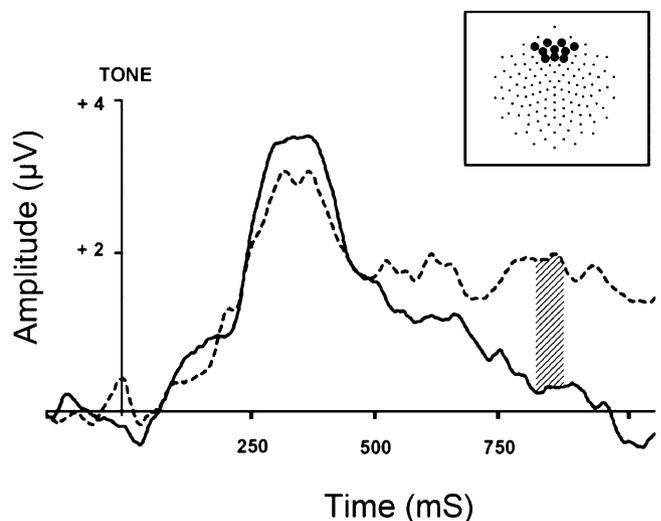


**Fig. 1.** (A) Sleeping newborn infant with the EEG net and air puff tube with an opening over the right eyelid. Speakers for tone presentation are located inside the pillows on either side of the infant's head. (B) The average probability of a conditioned eye movement response is shown for both the experimental and the control groups. In the experimental group, tones were immediately followed by a puff of air to the eye, whereas tones and air puffs were presented at random times in the control group.

the individual sensory AEPs showed no consistent differences in terms of either amplitude or latency for first and second halves of the experiment. In contrast to this sensory response, the late slow-wave ERP waveforms (792–842 ms) changed significantly during the experiment, with a more positive-going slow wave occurring in the second half of training compared to the first,  $F(1, 9) = 5.325$ ,  $P < 0.5$  (Fig. 2). This slow wave was measured from a group of 10 frontal leads where the effect was largest. It is presumed that this change in slow wave reflects cognitive processing associated with the tone stimulus after conditioning has occurred (20). However, because of the small amount of usable ERP data in the control group, it cannot be determined conclusively if the change was dependent on tone–puff pairing. It is possible that the slow wave reflects memory processing within the prefrontal cortex, as similar activity has been recorded in 3-month-old infants exposed to unfamiliar faces, presumably reflecting an updating of partially encoded memories (21). Alternatively, this positive wave may index the anticipation of the puff or even a preparedness to blink. The topographical distribution of the slow wave was not consistent with it being a direct reflection of the EMR per se, because its amplitude was not maximal closest to the eyes.

## Discussion

The current experiment demonstrates that newborn infants are capable of learning about relationships between stimuli while



**Fig. 2.** Event-related scalp potential (ERP) evidence of successful conditioning in newborn infant conditioning: a positive slow wave to conditioned stimuli in the second half of the training session (shaded area). The ERP elicited in the first half of the experiment is depicted by the solid line, and the ERP elicited by stimuli in the second half is represented by the dashed line. The earlier auditory evoked potential ( $\approx 300$  ms after tone onset) was present throughout training. The later positive slow wave activity ( $\approx 800$  ms after tone onset) recorded from frontal electrodes (Inset in upper right), was present only in the latter half of the training session.

asleep. Learning was demonstrated only in infants exposed to consistent pairings of the tone and air puff. In addition to a conditioned eye movement response, the newborns in the experimental group also showed a change in cortical activation. The change in amplitude was greatest over frontal areas, possibly indexing the updating of incomplete memories (21).

This capacity of infants to learn during sleep stands in contrast to the belief that learning of new material does not take place in sleeping adults (22), although this belief has yet to be tested using methods similar to the current study. It is possible that the greater plasticity of the brains of young animals lends itself to learning under circumstances that would be nonoptimal or prohibitive later in development (23). For example, immature organization of sleep states may be permissive with respect to neonatal learning. Newborns' sleep states are not well defined and become more organized over the first 2 years of life (24). Infants also differ from adults in their resting state networks during sleep, and it is thought that the default-mode network observed in adults may emerge gradually as the brain develops (25). Perhaps the opportunity for learning in nonawake states diminishes as sleep patterns mature over the course of development.

The ability to learn while asleep might be a very valuable adaptation during the neonatal period in terms of basic survival. Arousal deficits are the focus of considerable research into the mechanisms underlying sudden infant death syndrome (SIDS) (26–28). Mitchell et al. (29), in reviewing the SIDS literature, suggest that neonates who are inexperienced with prone sleep have to quickly learn how to escape potentially lethal situations in the face-down position. Specifically, adaptive conditioned arousal responses could be learned through pairings of cardio-respiratory challenges with postural cues (like those generated by sleep position) and/or environmental cues (such as a blanket on the face) (30, 31). Similarly, cues that predict food, warmth, or tactile stimulation during sleep might contribute to postnatal attachment (8) and Sambeth et al. (14) have shown that newborns process structural aspects of language while sleeping. Fetuses learn about structural aspects of maternal voice in utero (3, 4) despite

spending most of the time cycling within sleep states. Thus, the perinatal capacity to learn while asleep may play a pivotal role in the infant's rapid adaptation to the postnatal environment.

Eyelid conditioning relies on an intact functioning cerebellum (32), and functional MRI (fMRI) shows cerebellar activation during delay eyelid conditioning in adults (33). Thus, the current study demonstrates the feasibility of using eyelid conditioning as an early screen for cerebellar function, which may help identify infants at risk for several neurodevelopmental disorders. Functional cerebellar abnormalities are implicated in dyslexia, attention deficit hyperactivity disorder, autism, and schizophrenia (34, 35), and deficits in cerebellar mediated eyelid conditioning are associated with all of these disorders (36–41). Cerebellar abnormalities in neurodevelopmental disorders such as autism are thought to emerge prenatally (42). Moreover, the third trimester of pregnancy is a period of rapid cerebellar development, and preterm birth disrupts this process (43, 44). Because disruption of cerebellar development interferes with acquisition of the conditioned eye movement response (45), the conditioning paradigm described here offers a unique approach to non-invasive cerebellar-based assessment of developmental risk in newborn infants. Additionally, whereas delay eyelid conditioning does not necessarily depend on the cerebral cortex (46), changes in frontal activity associated with conditioning may provide early information about cortical function, as well.

## Materials and Methods

Thirty-four healthy term infants between 10 and 73 h of age were tested while asleep. After being fitted with cardio-respiratory sensors and a high-density EEG net, the infant was swaddled and placed supine in a bassinet. Tones were presented using two speakers, each placed next to the ears. A flexible tube was configured to deliver a puff of air to the outer canthus of the right eye (Fig. 1A).

Usable eye movement response data (without waking or crying or unacceptable amounts of electrical noise) were obtained from 30 infants (14 males and 16 females). Immediately following 3 tone-alone trials, 20 sets of 10

trials were presented to the experimental group ( $n = 26$ ). Each set of 10 trials consisted of eight tones paired with an air puff, 1 puff-alone trial, and 1 tone-alone trial. The puff-alone trials allow the unconditioned response to be monitored over the course of training without the influence of a prior tone. The tone-alone trials allowed the detection of conditioned responses unaffected by the presentation of an air puff on paired trials. The control group ( $n = 4$ ) was presented with the same number of stimuli as the experimental group in an equivalent amount of time but in a semirandom sequence.

The EMRs served as the primary measure of conditioned and unconditioned responses. Offline, the EMRs from four frontal electrodes along the eyebrow ridge were visually inspected during the 2000 ms after the conditioned stimulus onset on tone-alone trials and scored for the occurrence of a sharply rising electrical potential (20–200  $\mu$ V) in the midline lead nearest the eye (E17), indicative of muscle activation associated with eye movement (47). The onset latency of the EMR also was scored for tone-alone trials. For puff-alone trials, EMR was scored during the 1000 ms after puff onset.

EEG activity was recorded using a high impedance system with 124 electrodes spaced at 1-cm intervals over the scalp, using a vertex reference. Data were sampled at 1,000 Hz. All analyses were performed offline using a linked mastoid reference. The EEG in response to all tone stimuli (i.e., tone-puff pairs and tone-alone stimuli) was analyzed from 128 ms before stimulus presentation until 900 ms after tone onset (just before puff presentation on paired trials). Electrodes with artifact-free data for at least 80% of the epochs were included in the analysis (*SI Materials and Methods*). We examined if patterns of brain activation associated with early sensory or later cognitive processing (13, 21) changed over training. Because these patterns of activation are very small in relation to the background EEG, many responses need to be averaged to extract the stimulus-specific response from ongoing brain activity. Two averaged EEG waveforms (event-related potentials) were created for each infant on the basis of the first and second halves of the trials for both sensory and cognitive responses to the tone.

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