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Eyeblink classical conditioning and BOLD fMRI of anesthesia-induced changes in the developing brain

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HIGHLIGHTS

- Young adult rabbits were tested after exposure to anesthesia as infants.
- Acquisition of trace eyeblink classical conditioning was impaired.
- fMRI showed learning-related difference between control and anesthesia-exposed groups.

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ABSTRACT

Millions of children undergo general anesthesia each year in the USA alone, and a growing body of literature from animals and humans suggests that exposure to anesthesia at an early age can impact neuronal development, leading to learning and memory impairments later in childhood. Although a number of studies have reported behavioral and structural effects of anesthesia exposure during infancy, the functional manifestation of these changes has not been previous examined. In this study we used BOLD fMRI to measure the functional response to stimulation in the whisker barrel cortex of awake rabbits before and after learning a trace eyeblink classical conditioning paradigm. The functional changes, in terms of activated volume and time course, in rabbits exposed to isoflurane anesthesia during infancy was compared to unanesthetized controls when both groups reached young adulthood. Our findings show that whereas both groups exhibited decreased BOLD response duration after learning, the anesthesia-exposed group also showed a decrease in BOLD response volume in the whisker barrel cortex, particularly in the deeper infragranular layer. These results suggest that anesthesia exposure during infancy may affect the intracortical processes that mediate learning-related plasticity.

1. Introduction

Each year approximately 6 million children in the USA undergo anesthesia [9] with >1 million receiving multiple anesthesia exposures during the course of surgeries, imaging studies, and other diagnostic procedures [34]. There is increasing concern about the potential pathogenic effects of anesthesia on the developing brain, based on retrospective studies of children and adolescents exposed to anesthesia early in development [8,17,27,34,37]. Strikingly, one study reported that young children exposed to anesthesia were more than twice as likely to exhibit behavioral disorders or developmental deficits including mental retardation, autism and language or speech problems, in later years [10]. A growing body of literature from both human and animal studies has indicated that exposure to anesthesia, especially at an early age, can affect a variety of aspects of neuronal development, leading to deficits in learning and memory.

Animals studies have pointed to a variety of neuropathological changes that could account for the development of the learning and memory deficits observed in children [20]. For example, anesthesia produced cell loss in both the cortex and hippocampus in infant animals [12,15,16]. However, although a number of studies have identified structural changes that may link anesthesia and learning disability, the functional changes in the brain associated with these impairments have not been directly characterized. Previously, we have used blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) to examine the learning-related functional changes that occur during trace eyeblink classical conditioning (ECC) [24], an associative learning test that relies on the function of the cortex and hippocampus, in awake, behaving rabbits. We found that trace ECC was accompanied by a significant expansion of the BOLD activated volume in the cortex early in memory formation, demonstrating the effectiveness of fMRI in measuring functional changes associated with learning-related plasticity.

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In the present study we evaluated the effects of anesthesia exposure during infancy on trace ECC and functional activity in the somatosensory cortex, a region involved in mediating trace ECC [13], during young adulthood. Infant rabbits received isoflurane anesthesia and then underwent training with a trace ECC paradigm at three months of age. We performed fMRI experiments to measure the BOLD response to the whisker vibration before learning, in order to assess the effects of anesthesia on the whisker sensory system, and then after learning, in order to evaluate how behavioral learning modulated the sensory response for each group. Using awake rabbits allowed us to compare directly the results from rabbits exposed to anesthesia during infancy versus an unanesthetized control group, without the potentially confounding effects of additional anesthesia during imaging.

Considering the broad range of pathological effects reported to be associated with anesthesia, we hypothesized that the trace ECC learning rate would be significantly impaired in animals exposed to anesthesia during infancy compared to unanesthetized controls. Moreover, we hypothesized that differences between anesthetized rabbits and controls in terms of BOLD activated volume in the sensory cortex would occur in a learning-specific manner, and emerge only after training. Our results support previous behavioral studies which have reported behavioral learning deficits associated with anesthesia, and point to the need for a better understanding of the learning-specific functional changes that underlie these pathological effects, as well as the mechanisms that produce them.

2. Materials and methods

2.1. Animal preparation

Dutch-belted rabbits ($N = 10$) were used in accordance with the National Institutes of Health guidelines and NorthShore University HealthSystem Research Institute Institutional Animal Care and Use Committee approved protocol.

The rabbit kits were born in a nest box containing shredded aspen bedding which was prepared in advance. The newborn rabbit kits were housed and nursed with the dam until 4–6 weeks, which is the optimal weaning age for Dutch Belted rabbits. Beginning at postnatal day 8 the kits in the anesthesia group (2 females and 3 males) were anesthetized individually with a nose mask. Kits in the control group (3 females and 2 males) were exposed to the same environment as anesthesia group while breathing normal air. Maintenance of isoflurane concentration was controlled for 2 h with isoflurane at 1 MAC in air, using a scavenging canister on the exhaust side. The concentration was confirmed using a tail-clamping technique. Rabbits underwent anesthesia exposures on day 8 (mean of MAC is 1.6%), 11 (mean of MAC is 1.8%), and 14 (mean of MAC is 1.9%), for 2 h each exposure. During anesthesia the body temperature and respiration were checked every 15 min. A recirculating warm water heating pad (T-Pump, Gaymar Industries Inc., Orchard Park, NY, USA) was used to control temperature. The control group was placed in the same box for the same duration as anesthesia group. After each session rabbits were returned to the dam. Best efforts were made to prevent rejection of the rabbits by the dam (e.g., kits were wrapped in the nesting materials to maintain normal smell). None of the kits were rejected by the dam. 10 kits from 3 dams were used in these experiments.

At the age of three months animals were implanted with restraining headbolts and habituated to the MRI environment as described previously [1]. Animals were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg). 5–6 small bur holes were made in the skull without full penetration of the bone. Small nylon support screws were inserted into bur holes. A light-weight head restraining device containing four nylon bolts was implanted on top of the skull. This device also served to position the RF coil in the stereotaxic plane during MR experiments. The wound was completely covered with dental cement and if needed non-absorbable sutures were applied. After 1 week of recovery from surgery each subject was habituated for 3–5 days to the imaging environment prior to the experiments.

2.2. Eyeblink classical conditioning

ECC is a well-controlled test commonly used in both animals and humans in which the subject learns to associate a neutral conditioned stimulus (CS) with a behaviorally salient unconditioned stimulus (US). Rabbits were provided with earplugs during the experiments to reduce potentially distracting noise from the environment. The CS was delivered by deflecting two whiskers (A1 and B1) attached to a fiber band on the rabbit's left side at an amplitude of 1.5 mm and a frequency of 50 Hz, using a system described previously [19]. The US consisted of a 3 psi airpuff supplied by compressed air and controlled by a regulator and solenoid valve, using a system described previously [18]. The US was delivered to the left eye. Eyelid movements were measured with a fiber optic-based infrared reflectance sensor [23] which was positioned 1 cm from the cornea. The durations of the CS and US were 250 and 150 ms, respectively, with 500 ms stimulus-free trace interval. The total duration of a single trial was 8 s, with a 5–10 s random intertrial interval. A CR was defined as a change in the voltage from the detector that was 4 SD greater than the mean baseline amplitude and occurred at least 35 ms after onset of the CS but before the US. Eyeblink data were sampled at 300 Hz. Each subject received 1 session of conditioning trials per day for 10 days, where each session consisted of 100 trials. The animals were trained inside the magnet used for fMRI without the presence of noise from the pulsed field gradients. Following the training session on Day 10, the animals received 20 additional trials without attaching the whiskers to the fiber band in order to confirm that the subjects were conditioned only to the whisker stimulation and not to the sound of vibration. None of the rabbits showed >5% CRs for these trials.

2.3. fMRI stimulus delivery and data acquisition

In each experiment fMRI data were acquired from awake rabbits in response to whisker stimulation. Whisker stimulation was delivered by deflecting two whiskers (A1 and B1) at an amplitude of 1.5 mm and a frequency of 50 Hz using a system described previously [19]. Each animal received 10 trials of whisker stimulation, which consisted of a baseline (30 s), stimulation (20 s) and post-stimulus (40 s) period, before and after training with the ECC paradigm.

MRI experiments were performed on a 9.4 T imaging spectrometer (BioSpec 94/30USR, Bruker Biospin MRI GmbH) operating at 1 H frequency of 400 MHz. The spectrometer was equipped with actively-shielded gradient coil (BFG-240-150-S-7, Research Resonance, Inc., Billerica, MA, USA). A single-turn, 40 mm-diameter circular RF surface coil was used for both transmission and reception. Prior to each experiment, anatomical images were acquired using a multislice gradient echo pulse sequence with a TR of 1.5 s, a TE of 10 ms, a 30 mm × 30 mm FOV, and a matrix size of 128 × 128, corresponding to an in-plane resolution of 234 μm × 234 μm. fMRI data were acquired from four consecutive in the axial plane using a single-shot, gradient-echo multi-slice EPI sequence with a repetition time (TR) of 2 s, an echo time (TE) of 11 ms, a 30 mm × 30 mm field of view (FOV), and a matrix size of 80 × 80, corresponding to an in-plane voxel size of 375 μm × 375 μm, and a 1 mm slice thickness.

The fMRI data were corrected for small head motion using a 2-D affine registration method implemented using the Insight ITK toolkit [43] and subsequent processing was implemented in Matlab (The MathWorks, Inc., Natick, MA). Before averaging, the trials were inspected to assess the presence of any remaining head motion. No trials were excluded for these experiments. Trials were then averaged and activated voxels were detected in the averaged data using an unsupervised one-class support vector machine (SVM)-based algorithm, as described previously [32]. The SVM algorithm was applied without