Long-term effects of neonatal sevoflurane general anesthesia on rat hippocampally-mediated recognition memory

Allison Rowe, David Lempert, Tatiana Ramage, Jennifer Shih, Flora Chang, Kyle Barbour, Dana Ben-Tzur, Jeff Sall, Greg Stratmann

Department of Anesthesia and Perioperative Care, University of California, San Francisco

Abstract

Sevoflurane, a popular pediatric and veterinary general anesthetic, is thought to cause deficits in learning and memory as well as to increase neuronal apoptosis when administered to neonatal rodents (Satomoto et al. 2009). However, the mechanism and precise nature of sevoflurane-mediated memory deficits remain unclear. We hypothesized that neonatal sevoflurane anesthesia impacts recollection type recognition memory, which is mediated by the hippocampus, but not familiarity type recognition memory, which is mediated by the parahippocampal cortex. To test this, we administered sevoflurane to neonatal rats, trained them with a control group in recognition memory tasks, and are currently in the process of comparing the performance of the two groups using receiver operating characteristics, which statistically separate recollection and familiarity type memory. Once this analysis is complete, we will lesion the hippocampi of control group rats and continue testing their performance in memory tasks to see if any subsequent deficits renders their performance similar to anesthesia group rats. While our study is still in progress, results thus far indicate that, as hypothesized, recollection but not familiarity is impacted by neonatal sevoflurane general anesthesia, and thus that sevoflurane selectively impacts the hippocampus but not the surrounding parahippocampal cortex.

1. Introduction

All general anesthetics in current use are known to damage neuronal structure or to increase the likelihood of neurocognitive deficits when administered to neonatal rats (Loepke and Soriano 2008). Sevoflurane is one such general anesthetic in widespread human and veterinary use known to cause deficits in learning and memory and to trigger pervasive neuronal apoptosis when administered to neonatal rats (Satomoto et al. 2009). However, it is not known exactly which types of memory are impaired by sevoflurane, nor, as sevoflurane affects many structures, which specific structural damages cause the observed memory deficits. It is especially critical to clarify this further as it is possible that similar damage may occur after neonatal sevoflurane general anesthesia in humans.

Recognition memory can be broken up into two major classes: recollection and familiarity (Yonelinas 2002). Recollection-type memory involves active recall of specific prior encounters, whereas in familiarity, only a general feeling of prior interaction is experienced. Recent research indicates that recollection is mediated by the hippocampus, while familiarity is mediated by the surrounding parahippocampal cortex (Fortin et al. 2004, Sauvage et al. 2008). From preliminary work observing the performance of rats exposed to neonatal general anesthesia in a Morris water maze, we hypothesized that neonatal sevoflurane general anesthesia impairs recollection memory but not familiarity, and thus that neonatal sevoflurane general anesthesia impacts the hippocampus selectively without impacting the parahippocampal cortex.

To test this, we trained our rats to perform an odor recognition task and scored their results. A set of distinct odors would be presented individually to a rat to smell. After a delay, a second set of odors containing all of the original odors as well as novel odors in a random order would be individually presented. As we trained the rats to go to the back of their cage to receive a reward if they recognized the odor and to remain in the front if they did not, we were able to score their ability to recognize the odor. To gauge the certainty of a rat that it did or did not recognize an odor, we would bias the task on subsequent trials by making it harder to receive the reward in the front of the cage or by altering the reward amounts. We then analyzed our data using receiver operating characteristics (ROCs), a statistical method of analyzing memory tasks that distinguishes between familiarity and recollection type memory by relating the fraction of correctly recognized items to the fraction of incorrectly recognized novel items across different bias levels (Yonelinas and Parks 2007). This protocol is similar to that used by the Eichenbaum Laboratory of Boston University in their 2004 paper (Fortin et al. 2004) and was developed in collaboration with that laboratory.

Additionally, we will subject all animals to Morris water mazing after completing the ROC analyses. In this task, a rat is forced to swim in a pool of water until it finds a submerged platform upon which to rest as a way of testing spatial learning and memory (Morris 1981). We hypothesize that anesthesia group rats will take longer to find the platform as well as improve less rapidly over multiple iterations than control group rat as in our preliminary observations and in studies of rats with hippocampal lesions (Morris et al. 1982).

Once we have collected enough data to calculate ROC curves for all of our control rats and once Morris water mazing is complete, we will lesion the hippocampi of control rats bilaterally and resume ROC analyses to see if they too experience a recollection deficit without a familiarity deficit. If so, we will then have strong evidence that neonatal sevoflurane general anesthesia does cause a deficit in recollection memory but not in familiarity, and that that deficit is due to hippocampal damage. Additionally, we will also have strong evidence that neonatal sevoflurane anesthesia selectively impacts the hippocampus but not the surrounding parahippocampal cortex.

2. Materials and methods

Our experimental protocol follows the standards of the Institutional Animal Care and Use Committee of the University of California, San Francisco. On post-natal day 7, 10 male Sprague Dawley rats were randomly either administered 4 hours of 1 MAC sevoflurane or no anesthesia as a control, with 5 rats in each group. 8 weeks post-birth, rats were trained to dig in cups of sand to receive a food reward. Once they had mastered this task, rats would be presented with a cup of scented sand, then, after a delay, they would be presented with two cups of scented sand one after the other, one of which was the previously presented scent and the other of which was novel. The rats were trained to go to the back of their cage for their reward for the old scent and to dig in the cup for the novel scent.

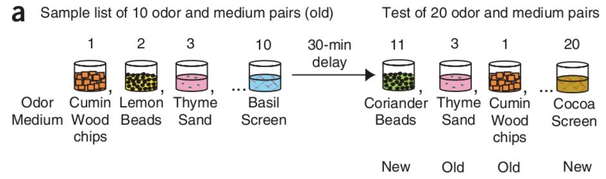

Figure 1: Example of the ROC testing protocol and the scented cups of sand used in both the training and testing phases. Note that the cups are reordered when presented for the second time. Figure from Sauvage et al. 2008.

Upon mastering this task, the rats moved into the ROC testing phase. During this phase, a rat would be presented with 11 scented cups of sand, one after another. Then, following a delay of 30 minutes, the rat would be presented with 22 scented cups of sand in a random order, 11 of which were the old scented cups. By making the cup taller or shorter, or by altering the rewards in the cup and the back of the cage, 5 bias levels were created as needed to calculate a ROC curve. The number of times that a rat went to the back of the cage correctly (hits) and incorrectly (false alarms) were scored. Once these ratios stabilized, the results from each bias level are averaged and then used to calculate the ROC curve.

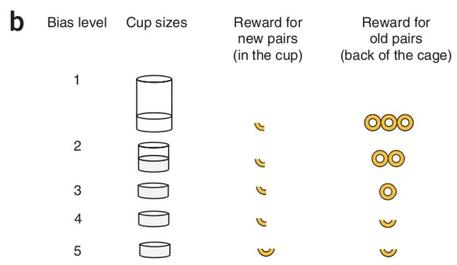

Figure 2: The five bias levels used in ROC testing. The cup size and rewards offered for new and old odors are varied to gauge a rat's certainty of recognition. Figure modified to match our experiment from Sauvage et al. 2008.

Morris water mazing will be conducted using a standard water maze. Lesions to the hippocampus will be done via stereotactic injection of a retrovirus bilaterally.

This experiment was conducted completely. However, our results did not meet our significance threshold. Therefore, we are currently repeating the experiment with an additional 13 rats to obtain more conclusive results.

3. Results and discussion

While we are still collecting data, our ROC analyses thus far indicate that anesthesia group rats use only familiarity memory, while control rats use both familiarity and recollection memory. Results from Morris water mazing remain inconclusive. Lesion studies have not yet been performed successfully, although results are expected within several months.

Assuming that our ROC analysis results continue to be accurate once we collect more data, we will be able to conclude that neonatal sevoflurane general anesthesia leads to a long-term deficit in recollection memory, but not familiarity memory, in rats. This implies that sevoflurane damages the hippocampus but not the parahippocampal cortex. It is currently unclear what implications this will have for both veterinary and clinical human anesthesia, although such deficits must be further investigated if present.

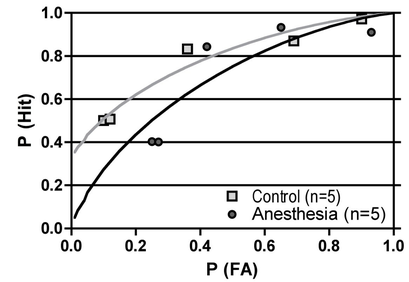

Figure 3: An example ROC curve showing loss of recollection in anesthesia group rats (seen in the *y*-intercept being near 0), retention of recollection in control group rats (seen in the large positive *y*-intercept), and retention of familiarity in both groups (seen in the high curvilinearity). The fraction of false alarms is represented on the *x*-axis and the fraction of hits is represented on the *y*-axis. The 5 data points plotted per group represent the average value from each of the 5 bias levels, with bias level 1 on the far right and bias level 5 on the far left. Figure courtesy Allison Rowe.

4. Acknowledgments

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