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Olfactory Behavior in ApoE Knockout Mice

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Olfactory Behavior in ApoE KO Mice

(TITLE)

BY

John P. Yost

1977-

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

2001
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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OLFACTORY BEHAVIOR IN APOE KNOCKOUT MICE

Master's Thesis

By
John Yost

Acknowledgements

I would like to sincerely thank my friends, family and research colleagues, especially Yanwen Jiang, Sophia Shen, Emily Rosario, Matthew Kircher, and most importantly, my wife, Shannon. Without the camaraderie, ingenuity, and support of these amazing people, I could not have completed this thesis. I want to thank my advisor and mentor, Dr. Nathan, who patiently offered constant encouragement and novel solutions to infinite obstacles during the last two years. I am grateful that he took the time to introduce me to the world of professional research. I would also like to thank my thesis committee members, Dr. Fritz who helped me painstakingly engineer a functional PCR system, and Dr. Laursen who helped me with many immunological techniques in my initial exploratory experiments. I must also thank Dr. Marjanovic and Dr. Mcgilliard for their generosity in supplying the PowerLab, and their many helpful technical suggestions. Finally, thanks to Dr. Switzer for his help with statistical analysis of more than 900 pages of raw data.

Table of Contents

Abstract:

Apolipoprotein E (apoE), a lipid transporting protein, has been shown to play a vital role in nerve repair and remodeling. Since the olfactory system is in a continuous state of remodeling, the present study tested the hypothesis that apoE is required for normal functioning of the olfactory system. Olfactory function of the wild type (WT) and apoE deficient (apoE KO) mice was assessed by using three standard olfactory tests: 1) the buried food pellet (BFP) test; 2) the odor choice (OC) test; and 3) the odor cued taste avoidance (OCTA) test. The visible food pellet test (VFP) was not used to assess olfactory function, but to rule out lack of motivation to find a buried pellet. ApoE KO mice performed poorly in all the tests as compared to WT mice, except the visible pellet test, in which the apoE KO mice performed better than wild type. ApoE KO mice had significantly longer latency to find the buried pellet than WT mice in the BFP test. In the OC experiment, apoE KO mice were unable to differentiate water from an odorant solution. Furthermore, in the OCTA test, the apoE KO mice were significantly less effective than WT mice at avoiding water containing an odorant and tastant. These data demonstrate that apoE deficiency in apoE KO mice leads to a deficit

in olfactory function, suggesting an important role for apoE in the olfactory system.

Introduction:

ApoE is a 34-kDa protein component of lipoproteins that functions in the redistribution of lipids among cells of various organs (Mahley, 1988). It is primarily synthesized in the liver but is also expressed in significant amounts in the brain (Elshourbagy et al., 1985). ApoE levels increase in both the central and peripheral nervous system following crush injury, and have been proposed to scavenge lipids from the degenerating neurons for recycling to growth cones of sprouting axons (Poirier, 1994). This is important in both maintenance and repair of the nervous system. Humans have three major isoforms of apoE (apoE2, apoE3, and apoE4) that are produced by three alleles (e2, e3, and e4) at a single gene locus on chromosome 19 (Das et al., 1985; Mahley, 1988; Zannis et al., 1982). The most common isoform is apoE3, which contains cysteine and arginine at positions 112 and 158, respectively (Weisgraber, 1994). Both positions contain cysteine in apoE2 and arginine in apoE4. Mice have one form of apoE which is "apoE3-like" in its function (Weisgraber, 1994).

Recently, there is a surge of interest in apoE in neurobiology primarily due to the finding that the E4 allele is a dose-dependent risk factor for Alzheimer's disease (AD) (Corder et al., 1994; Corder et al., 1993; Poirier et al., 1993). Furthermore, AD patients with the

apoE4 allele usually showed an earlier age of onset and a more rapid progression of the disease. Also, apoE localizes in neurofibrillary tangles and amyloid plaques, the two characteristic lesions in AD brains (Namba et al., 1991; Schmechel et al., 1993). These findings demonstrated a link between apoE polymorphism and the development of AD; however, the mechanism of this relationship is still unclear.

Studies have consistently shown olfactory dysfunction in patients with AD (Murphy, 1999). AD patients with moderate dementia have deficits in odor identification, detection, threshold, and memory (Doty et al., 1987; Murphy et al., 1990; Nordin and Murphy, 1998; Serby et al., 1991). Furthermore, AD patients show neuropathological changes in components of the brain involved in olfactory processing (Braak and Braak, 1997; Braak and Braak, 1994; Davies et al., 1993; Struble and Clark, 1992; Talamo et al., 1989). In fact, olfactory deficit is one of the first signs of AD, and has been proposed as a predictor of the disease (Bacon et al., 1998; Devanand et al., 2000; Graves et al., 1999; Morgan et al., 1995; Nordin and Murphy, 1996). Interestingly, apoE allele status is also associated with olfactory dysfunction (Bacon et al., 1998; Graves et al., 1999; Murphy et al., 1998). Recent studies have demonstrated that normal elderly persons with the apoE4 allele showed significantly poorer

odor identification than those lacking the apoE4 allele (Murphy et al., 1998). Furthermore, in individuals with mild cognitive impairment, those having the apoE4 allele showed poorer odor threshold than those without this allele (Murphy, 1999). Taken together, these data suggest that apoE plays an important role in olfactory function.

We have previously reported that apoE is expressed at high levels in human and mouse olfactory bulbs, particularly in the olfactory nerve layer and around the glomeruli (Struble et al., 1999). In a later study we showed that apoE deficiency in apoE-gene deficient/knockout (apoE KO) mice leads to substantial delay in olfactory nerve repair (Nathan et al., 2000). In the present study, we hypothesized that apoE KO mice should have a deficit in olfactory function. We assessed the performance of apoE KO mice in three different tests of olfaction. The results from this study revealed that apoE mice are defective in olfactory functioning.

Methods and Materials:

Animals: ApoE KO (C57BL/6J-Apoe ^{tm1Unc}) and wild-type (WT) C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were housed in a sound-attenuated room under constant temperature (22 ^{o}C), light from 4 AM to 4 PM and access to water and food *ad libitum*. Mice were housed individually. Male mice, two to four months old, were used in the experiment. All mice were acclimatized to the sound-attenuated environment and 12 hour, fluorescent light schedule for a period of seven days prior to any testing. To avoid diurnal variation each mouse was tested at the same time of the day. A single blind procedure was used in all experiments so that the experimenter was unaware of the genotype of the animals used in the study.

Buried Food Pellet Test: A modification of the buried food pellet (BFP) test by Edwards et al, 1972, was employed except Purina mouse chow pellet (Lab Diet, Mouse Diet 9F, 5020. Catalog No. 04CMS-5020. PMI Nutrition International, Inc.) was used instead of an exogenously scented pellet. This modification avoided the need for pre-training the mice to associate exogenous odor to food. Mice were placed on a food restricted diet (0.2g chow/mouse/24 h) starting from two days prior to testing and during the 3-day experimental period.

On each of the 3 testing days mice received one trial per day. In each trial a single mouse was placed at random in a test cage (45cm x 24cm x 20cm) to recover a 1-gram food pellet buried approximately 0.5 cm below the surface of a 3 cm deep layer of mouse bedding material. The location of the food pellet was changed daily in a random fashion. The latency to find the food pellet was defined as the time it took the mouse to uncover the food pellet and grasp it in its forepaws and/or teeth. Animals were allowed to consume the pellets they found and were then returned to their home cage. An animal that did not find the food pellet within 5 min was removed and placed back into its home cage. The bedding in the test chamber was changed between trials.

Visible Food Pellet Test: A modification of the buried food pellet (BFP) test by Edwards et al was employed except that the mouse chow pellet was visible during the testing period. The administration of the visible pellet test is otherwise identical to the BFP.

Odor Choice Experiment: The odor choice (OC) test used in this study was similar to that described for rats by Darling & Slotnick (1994). The test chamber consisted of a metal-floored box (20cm x 30cm x 12cm) fitted with two 10 ml syringes each with a stainless steel drinking nozzle (0.7 cm outside diameter). One syringe

contained tap water (S+), and the other syringe contained 0.1% isoamyl acetate (ICN Biomedicals Inc. Catalog No. 155077.) in tap water (S-). Darling and Slotnick have previously demonstrated that rodents exhibit an aversion to the odor of isoamyl acetate. The nozzles of the syringes and the metal floor were connected to a Powerlab (PowerLab, Model 400. ADI Instruments Pty. Ltd.) by an electrical cable (Human Physiology Teaching Set. Model K01-1199. CB Sciences.). Thus, a touch circuit was established that was measured by Gateway E-4200 PC computer using Chart (Chart version 3.46, software. ADI Instruments Pty. Ltd.) software. This computerized touch circuit precisely measured the latency to first contact.

Twenty-four hours before testing, mice were deprived of water. At the end of the deprivation period mice received one trial, in which each mouse was placed in the center of the testing chamber, facing the wall between the two nozzles, and allowed free access to the nozzles for 5 minutes. A positive drink score was awarded if a mouse made 0.5 seconds of sustained oral contact with a nozzle. A mouse that received a positive drink score was immediately removed from the testing chamber. The aluminum foil floor of the test cage was changed between the testing of each mouse.

Odor Cued Taste Avoidance experiment: The odor cued taste avoidance (OCTA) test is a modification of previously published procedures (Darling and Slotnick, 1994; Liebenauer and Slotnick, 1996). The test chamber used in the OCTA test was similar to that described in the odor choice test except the box had a single opening through which the stainless steel metal drinking nozzle, fitted with the 10 ml syringe, could be placed at variable distances from the opening. Furthermore, a PVC tube was attached over the nozzle such that the nozzle slid freely in the PVC tube. The computerized touch circuit was similar to that described in odor avoidance test.

Mice were placed on a 0.4 ml/day water-deprivation schedule that started twenty-four hours before testing, and continued throughout the 6-day test schedule. Each trial consisted of two periods: 1) A sampling period of 30 seconds in which the nozzle end was recessed 1.5cm into the PVC tube such that the mice could sniff the odor from the nozzle, but could not make contact with the nozzle, and 2) a drinking period of 60 seconds in which the nozzle was moved forward so that the mice could contact the drinking nozzle. A positive contact score was awarded upon any contact with the nozzle, and the mouse was immediately removed from the testing chamber. Trials were separated by a 30-second rest period during which the mouse was placed in a separate cage, with no access to food or water.

The aluminum foil flooring of the test chamber was changed between each mouse. At the end of the sixth trial, the mouse was returned to his home cage that contained 0.4 ml water and food *ad libitum*.

Each mouse received 6 trials/day for 6 consecutive days. During days 1-3, mice were trained to drink tap water from the nozzle. During days 4 and 5, animals were offered three trials with S+ solution (water) and three trials with S- solution, 0.1% vanillin (Acros Organics, Catalog No. 140822500), and 0.05% quinine monohydrochloride dihydrate (QHCl, Acros Organics, Catalog No. 163720250). The order of S+ and S- was randomized within a day so that each individual received the same random order within a day but the sequence of S+ and S- differed among days. On day 6, the randomly organized 6 trials continued, however, the S- concentration of vanillin was reduced from 0.1% to 0.001%, while the concentration of QHCl remained the same. Vanillin was used as an odorant because it is solely an olfactory nerve stimulant and is not likely to stimulate the trigeminal nerve. By using vanillin, the experimenter is assured that any odor cued taste avoidance is due to ability to detect an odor, and is not due to a trigeminal reflex.

Results:

Buried Food Pellet Recovery: In order to investigate whether WT and apoE KO mice differed in their ability to find buried food pellets, a 2 factor ANOVA with day and mouse strain as factors and latency as the dependent variable was performed. Both mouse strains found the pellets more quickly over time ($F_{2, 153} = 40.99$, $P < 0.0001$), but KO mice were significantly slower at finding buried pellets than wild type mice, on all three test days, ($F_{1,153} = 33.33$, $P < 0.0001$) (Fig. 1). The interaction between genotype and day was not significant ($F_{2,153} = 1.25$, $P = 0.29$), indicating that mice from both genotypes improved over time in their ability to find buried food pellets in a similar manner. The results of the BFP test indicate that WT mice are faster at recovering a buried pellet than are KO mice (Fig. 1).

Visible Food Pellet recovery: The result from the visible pellet test was dramatically different than that from the BFP test. In the visible pellet test the apoE KO mice were actually faster than wild type mice at finding the pellet (KO mean ± SE = 34.9 ± 4.2 sec, wild type = 58.9 ± 7.2; $t = 2.88$, $P = 0.01$), suggesting that KO mice were motivated to find food. The results of the VFP test indicate that apoE

KO mice are faster at recovering a visible pellet than are wild type mice (Fig. 2).

Odor Choice Experiment: When given a choice between a bottle containing only water and a bottle containing water and 0.1% isoamyl acetate, WT mice strongly preferred the plain water. Seven of seven WT individuals (100%) contacted (i.e. maintained contact for 0.5 s) water first (X_2 = 7.0, df = 1, P < 0.01) (Fig. 3 and 24-27). In contrast, 7/12 (58%) of apoE KO mice contacted water first, which is not significantly different than random (X_2= 0.33, df = 1, P > 0.50). For those apoE KO individuals that contacted both bottles, latencies until contact with water were not significantly shorter than those for odor (N = 11; water: 104.2 ± 18.2 s, odor: 105.4 ± 28.9 s; paired t = 0.05; P = 0.96) (Fig. 4).

Odor Cued Taste Avoidance: We examined the initial 3 day training period, consisting only of the S+ (water) treatment, to see if wild type and KO mice differed in their ability to perform the one bottle task. A repeated measures ANOVA model, including the effects of genotype and day, explained a significant portion of the variance in latency (whole model R^2_{adj} = 0.30, $F_{18,287}$ = 8.43, P< 0.0001) (Fig. 5-10). Wild type and KO mice did not differ in their latencies across the training period ($F_{1,287}$=0.30, P = 0.59), but a

significant decrease existed across days ($F_{2, 287} = 10.0$, $P < 0.0001$) and consistent differences existed among individual mice ($F_{15,287} = 8.62$, $P < 0.0001$). Thus, the latencies for both wild type and KO mice both decreased, and decreased at the same rate; this suggests that wild type and KO mice did not differ in their ability to learn this task.

In order to examine whether wild type and KO mice responded to the vanillin-cued quinine, within-individual comparisons for wild type and KO mice separately for day 4 and 5 (vanillin 1% + QHCl 0.05%) and day 6 (vanillin 0.001% + QHCl 0.05%) trials were first conducted. Wild type mice had significantly longer latencies to S- than S+ for all 3 days (Fig. 6). ApoE KO mice only had significantly longer latencies on day 5. When vanillin concentrations were lowered on Day 6, the trend between S+ and S- treatments was non-significant, suggesting that the KO mice had more difficulty detecting the vanillin than did wild type mice. The significant effect on day 5, however, suggests that KO mice were capable of making the association between the odor and the taste stimuli.

Several lines of evidence suggest that wild type and KO mice differed in their ability either to detect the vanillin or associate the vanillin with the quinine. First, wild type mice had significantly longer mean S- times than KO on all three days (day 4: WT = 38.9 ± 4.7, KO 14.0 ± 3.7; $t = 5.3$, $P < 0.0001$; day 5: WT = 44.9 ± 4.1, KO =

19.5 ± 4.6; t = 4.79, P = 0.0002; day 6: WT = 38.7 ± 4.6, KO = 9.9 ± 2.0; t = 8.9, P < 0.0001, Fig. 5). Second, the mean treatment difference (i.e. s- - s+) between wild type and KO mice indicates that a larger difference exists for wild type mice on all three test days (day 4, t = 3.47, P = 0.003; day 5, t = 3.62, P = 0.002; day 6, t = 7.6, P < 0.0001), with the largest difference occurring on day 6, the day with the lowest vanillin concentration. Third, 7/8 wild type versus 0/9 KO mice did not drink during at least one of their 3 S- trials on day 6 (Fisher's exact test, P = 0.0004). Fourth, on the first test day (i.e. day 4), the first trial was S+ and the second trial was S-. Therefore, we compared wild type and KO in their response to S- at their first exposure. Five of 8 WT mice had longer latencies with S- than with S+, while all 9 KO mice had longer latencies with S+ (Fisher's exact test, P = 0.009). This longer latency for wild type with S- was unlikely to be due to them satiating their thirst; e.g. on the previous day (day 3, with all trials S+), 0/8 wild type individuals had longer latencies with their second trial than their first.

The results of the odor cued taste avoidance test indicate that both the WT and apoE KO mice are able to associate the quinine taste with the vanillin odor. However, the WT mice are significantly more likely to avoid the vanillin odor than are the apoE KO mice, likely

indicating that the WT mice were better able to detect the vanillin odor, at any concentration, than are the apoE KO mice.

Discussion:

The results from this study demonstrate that apoE deficiency in apoE KO mice leads to a deficit in olfactory function. ApoE KO mice performed poorly in all three tests used to evaluate olfactory function as compared to WT mice. Although, these data could be interpreted as olfactory functional deficit in apoE KO mice, there are other possible alternative interpretations. First, it is possible that apoE KO mice have motor impairments that could have contributed to the delay in finding the buried food in BFP test or drinking solutions in the OC experiment. However, previous studies did not find any significant differences between apoE KO and WT in locomotor activity (Lominska et al., 2001; Oitzl et al., 1997). Furthermore, in the visible pellet test apoE KO were faster than WT mice in finding the pellet. Also, the latency to drink water in the first 3 days (training period) of OCTA test of apoE KO mice and WT were not significantly different from each other. These data suggest that apoE KO mice have normal locomotor function. Second, hunger/thirst level or motivational properties of the animals could influence performance on appetitive tests like those used in this study. Several lines of evidence, however, suggest that apoE KO animals were motivated to seek food and water in our experiments. For example, in the OC and

the OCTA tests the latency to drink water of apoE KO and WT were approximately equal. Also, in the visible pellet test apoE KO mice performed better than WT in finding the pellet. Furthermore, Raber et al. (2000), have recently reported that 6 month old apoE KO mice did not differ from WT mice of the same age in food or water intake (Raber et al., 2000). Taken together these data strongly argue against the lack of motivation or hunger/thirst as the primary cause for olfactory deficit in apoE KO. Third, inability to learn a task could have contributed to poor performance in olfactory tests used in this study. In our studies we did not observe any dramatic cognitive deficit in apoE KO mice, even though our experiments were designed to specifically test olfactory function and not cognitive behavior. For example, in the initial 3 days training period of OCTA test with S+ alone (water), the latencies of both apoE KO and WT significantly decreased at the same rate across days, suggesting that mice from both genotypes did not differ significantly in their ability to learn this task. Taken together results from this study revealed that apoE KO mice have a deficit in olfactory functioning, suggesting an important role for apoE in the olfactory system.

The function of apoE in the olfactory system is unclear, however, a series of observations suggests that apoE may play a vital role in this system. Grehan et. Al. have demonstrated exceptionally

high levels of apoE in the olfactory bulb. ApoE mRNA was particularly prominent in astrocytes in the glomerular layer of the bulb. We have previously observed that apoE is found in the mouse and human olfactory bulbs specifically in the olfactory nerve layer and around the glomeruli (Struble et al., 1999). Furthermore, apoE level in the olfactory bulb increases concurrent with olfactory nerve lesioning in mice (Nathan et al., 2000). Double labeling immunocytochemical studies revealed that both reactive astroglia and microglia contributed to the increase of apoE following olfactory lesioning. In a recent study that examined the effects of apoE deficiency on olfactory nerve recovery following peripheral receptor lesion we found that olfactory nerve regeneration is delayed in apoE KO mice as compared to WT mice (Nathan et al., 2000). Thus one potential role for apoE in the olfactory system is to support the perpetual degeneration and regeneration of neuronal terminals that occurs in the olfactory bulb as a result of the continuous turnover of the receptor neurons in the olfactory epithelium.

The importance of apoE in the olfactory system is substantiated by human studies that have examined the effects of apoE isoforms on olfactory function. Results from these studies revealed that individuals who were anosmic and possessed an apoE4 allele were at significantly higher risk for cognitive decline than

normosmics with out an apoE4 allele (Graves et al., 1999). Also, AD patients positive for apoE4 allele have impaired odor identification (Murphy et al., 1998). Furthermore, in individuals with mild cognitive impairment, those with the apoE4 allele showed higher odor thresholds than those without the apoE4 allele (Bacon et al., 1998). Taken together these data suggest that apoE4 may not support the continuous neuronal remodeling in the olfactory system as efficient as the other isoforms of apoE, and thus may lead to olfactory dysfunction.

To our knowledge this is the first study to document olfactory dysfunction due to apoE deficiency in mice. Several previous studies have found cognitive deficits in apoE KO mice; however, there are data to the contrary (Anderson et al., 1998; Gordon et al., 1996; Gordon et al., 1995; Krzywkowski et al., 1999; Oitzl et al., 1997; Puolivali et al., 2000; Raber et al., 1998; Veinbergs et al., 1998; Zhou et al., 1998). Given our finding that apoE KO mice exhibit an olfactory deficit, these findings have to be carefully interpreted. For example, poor performance of apoE KO mice in a cognitive test where odor-emitting rewards, like food, are used could be misinterpreted as a cognitive deficit, when it is most likely due aberrant olfactory capability.

Legend of Figures:

Figure 1. Performance of WT and apoE KO on buried pellet test. On each of the first 3 consecutive days, the latency for the mice to recover a buried food pellet was recorded. Latency declined significantly across days in both apoE KO and WT mice. However, latency of apoE KO mice was significantly higher than WT on all the three days ($F_{1,153} = 33.33$, $P < 0.0001$*).

Figure 2. Performance of WT and apoE KO on visible pellet test. ApoE KO mice are faster at retrieving a visible pellet than wild type mice. Latency to find a visible pellet was recorded. In contrast to buried pellet test, apoE KO mice had significantly shorter latency to find the visible pellet as compared to WT mice. (KO mean ± SE = 34.9 ± 4.2 sec, wild type = 58.9 ± 7.2; $t = 2.88$, $P = 0.01$) *$p < 0.01$, $p < 0.001$ versus WT.

Figure 3. Percentage of WT and apoE KO mice that contacted water first in the odor choice experiment. Mice were presented with a choice of 0.1% isoamyl acetate or water, and the solution they contacted first was recorded. The number of mice in the apoE KO group (58%) that contacted water first was significantly lower than those from the WT group (100%). * $p < 0.01$, $p < 0.001$ versus WT.

Figure 4. Latency of apoE KO mice to contact water and odor bottles in odor choice experiment. ApoE KO mice were presented with a choice of 0.1% isoamyl acetate and water, and the latency to contact the solutions was recorded. Latency to contact water and 0.1% isoamyl acetate were similar in the apoE KO mice. (N = 11; water: 104.2 ± 18.2 s, odor: 105.4 ± 28.9 s; paired t = 0.05; P = 0.96)*

Figure 5. Performance of WT and apoE KO mice on odor cued taste avoidance task. In the initial 3-days training period, with only S+ (water) used, there were no significant differences between apoE KO and WT in the daily latency value ($F_{1,287}$=0.30, P = 0.59). However, latency of both WT and apoE KO mice significantly decreased across days ($F_{2, 287}$ = 10.0, P < 0.0001). Also, on the 3-days testing period, the latency of both WT and apoE KO mice continued to decrease across days. There were no significant differences between apoE KO and WT mice in their latencies to S+ (water). However, apoE KO mice had significantly shorter latency to S- than WT mice. *p < 0.01, p< 0.001 versus WT.

Figure 6. Mean treatment differences [(S- latency) – (S+ latency)] between WT and apoE KO mice in odor cued taste avoidance task. Mean treatment differences were significantly lower in the apoE KO mice than WT on all the three testing days (day 4, t =

3.47, P = 0.003; day 5, t = 3.62, P = 0.002; day 6, t = 7.6, P < 0.0001).

* p < 0.01, p< 0.001 versus WT.

Figure 7. Raw data image for OCTA test wild type mouse latency on day 1 with S+. Example of no contact, WT.

Figure 8. Raw data image for OCTA test apoE KO mouse latency on day 1 with S+. Example of no contact, apoE KO.

Figure 9. Raw data image for OCTA test wild type mouse latency on day 2 with S+. Example of contact, WT, approx. 30 seconds to contact.

Figure 10. Raw data image for OCTA test apoE KO mouse latency on day 2 with S+. Example of contact, apoE KO, approx. 25 seconds to contact.

Figures:

Figure 1. Performance of WT and apoE KO on buried pellet test.

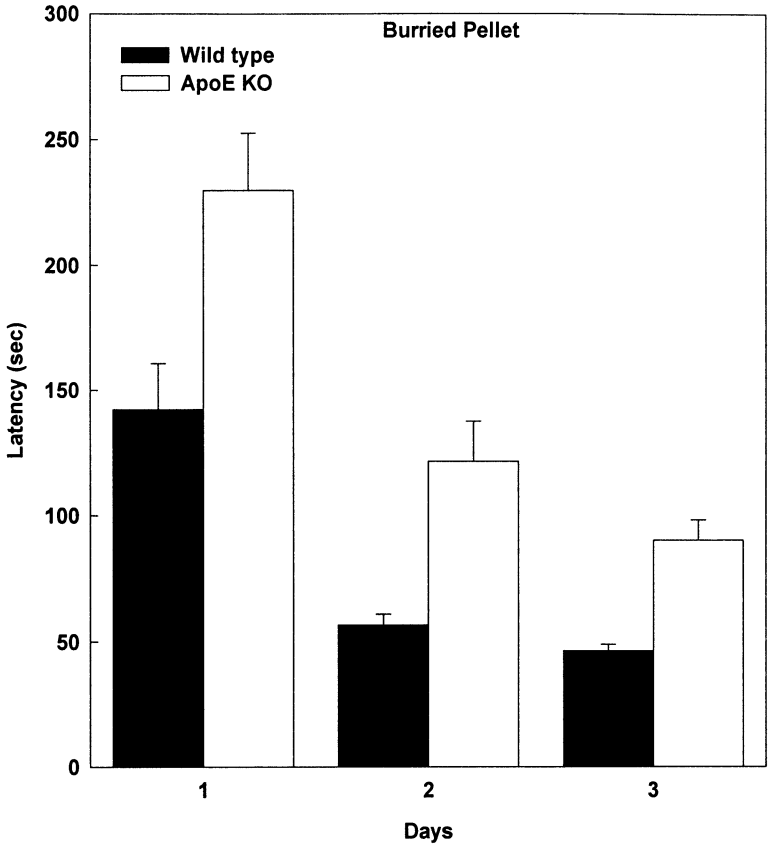


Figure 2. Performance of wild type and apoE KO on visible pellet test

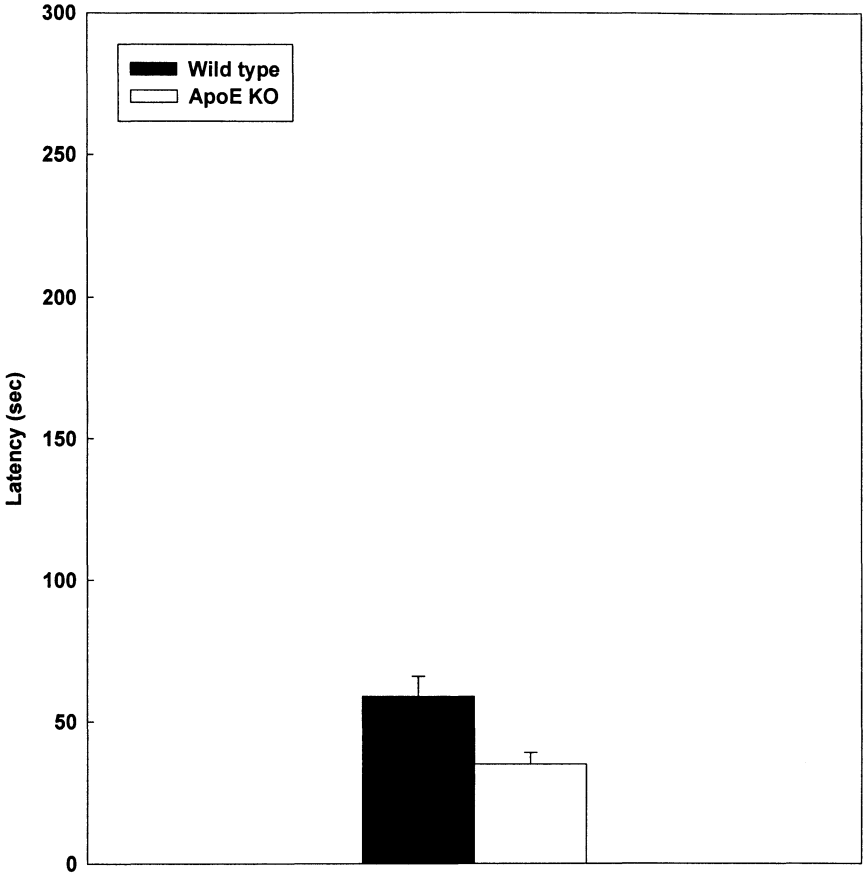


Figure 3. Percentage of WT and apoE KO mice that contacted water first in the odor choice experiment.

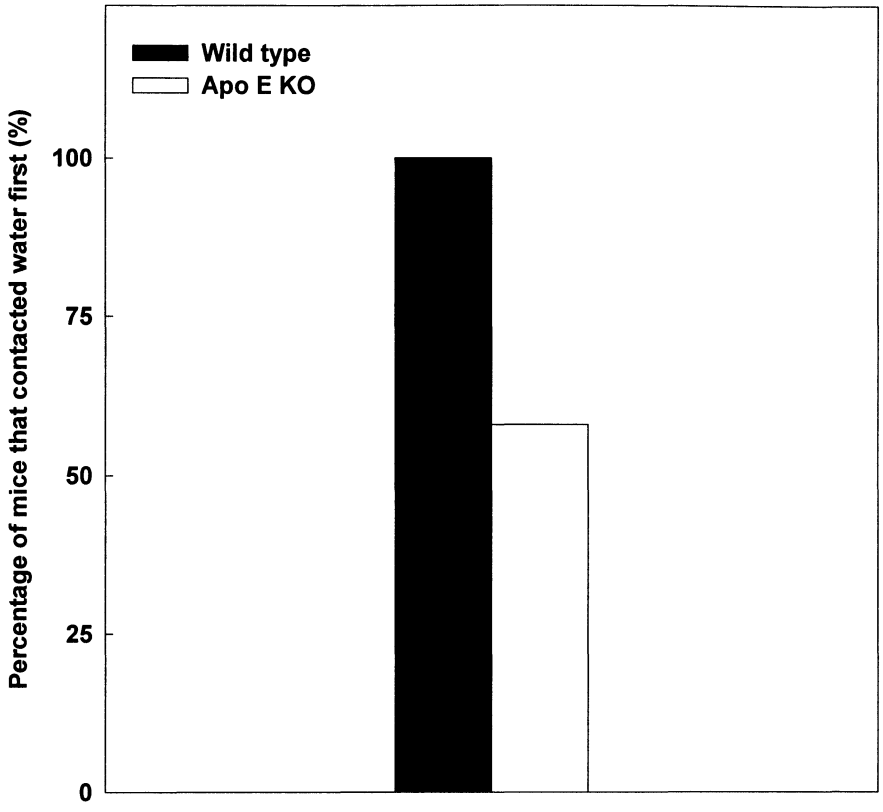


Figure 4. Latency of apoE KO mice to contact water and odor bottles in odor choice experiment.

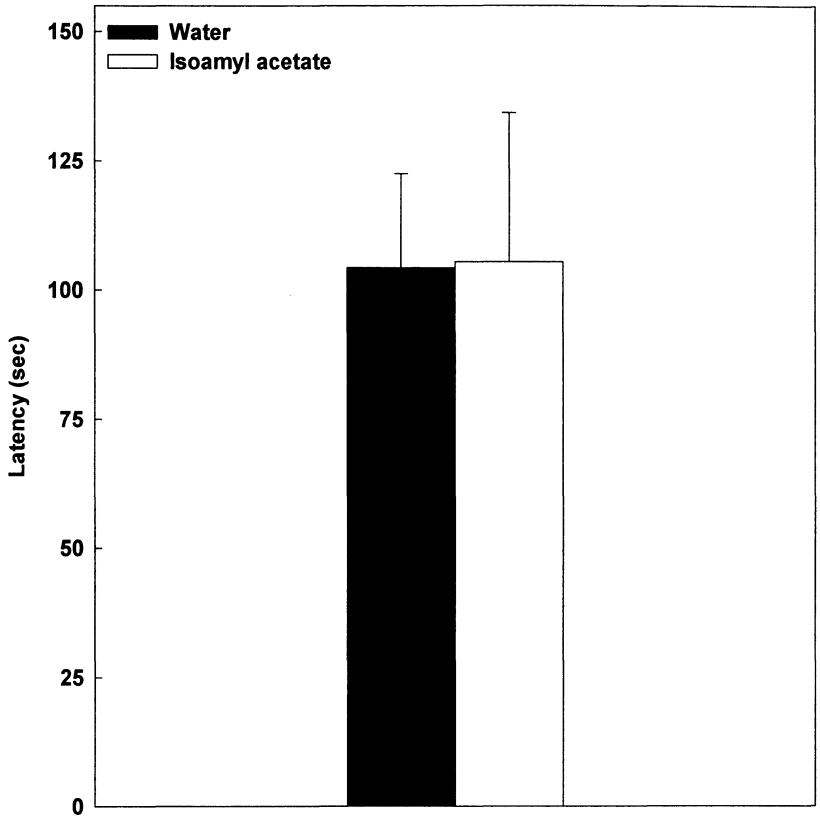


Figure 5. Performance of WT and apoE KO mice on odor cued taste avoidance task.

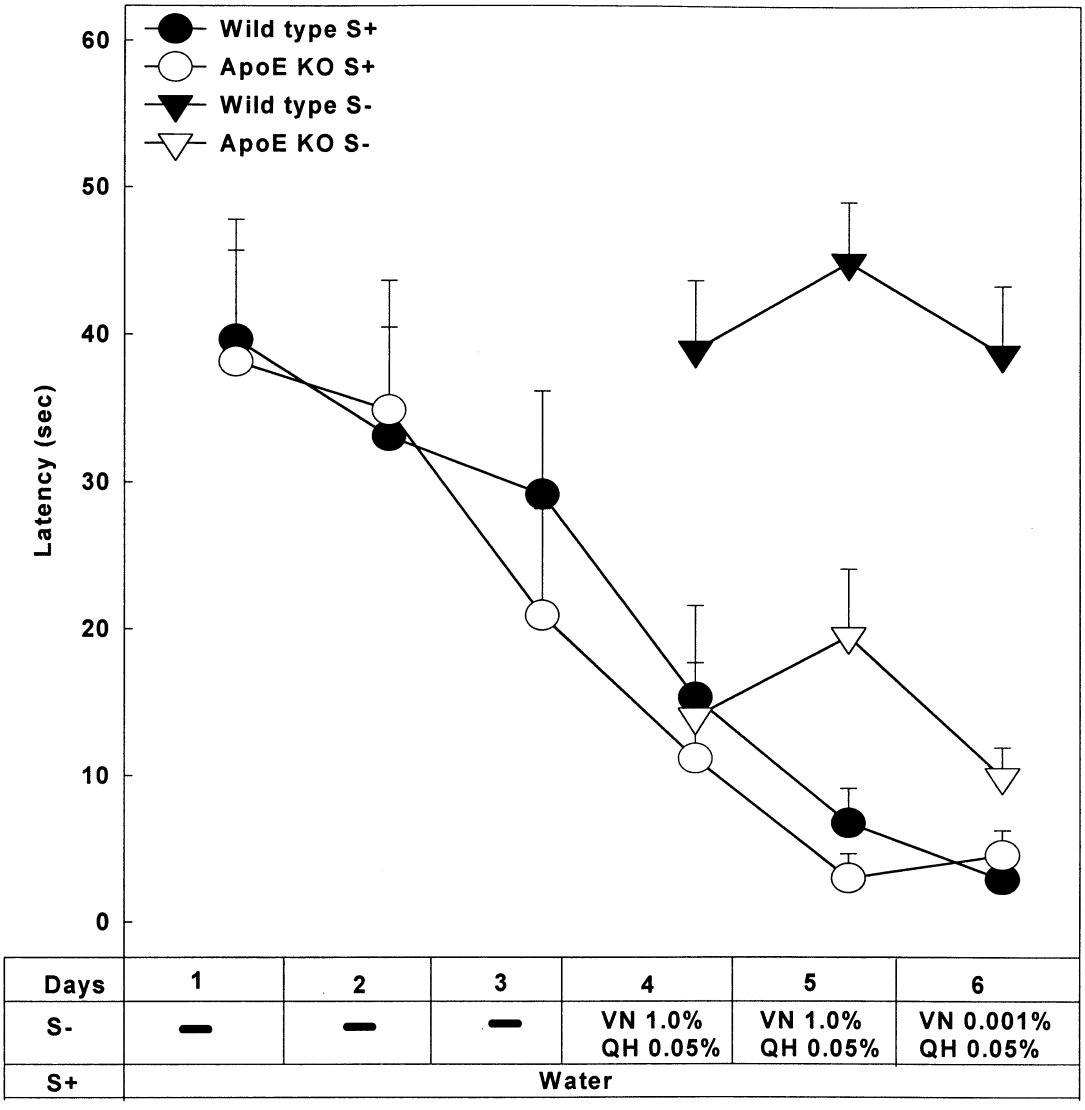


Figure 6. Mean treatment differences [(S- latency) – (S+ latency)] between WT and apoE KO mice in odor cued taste avoidance task.

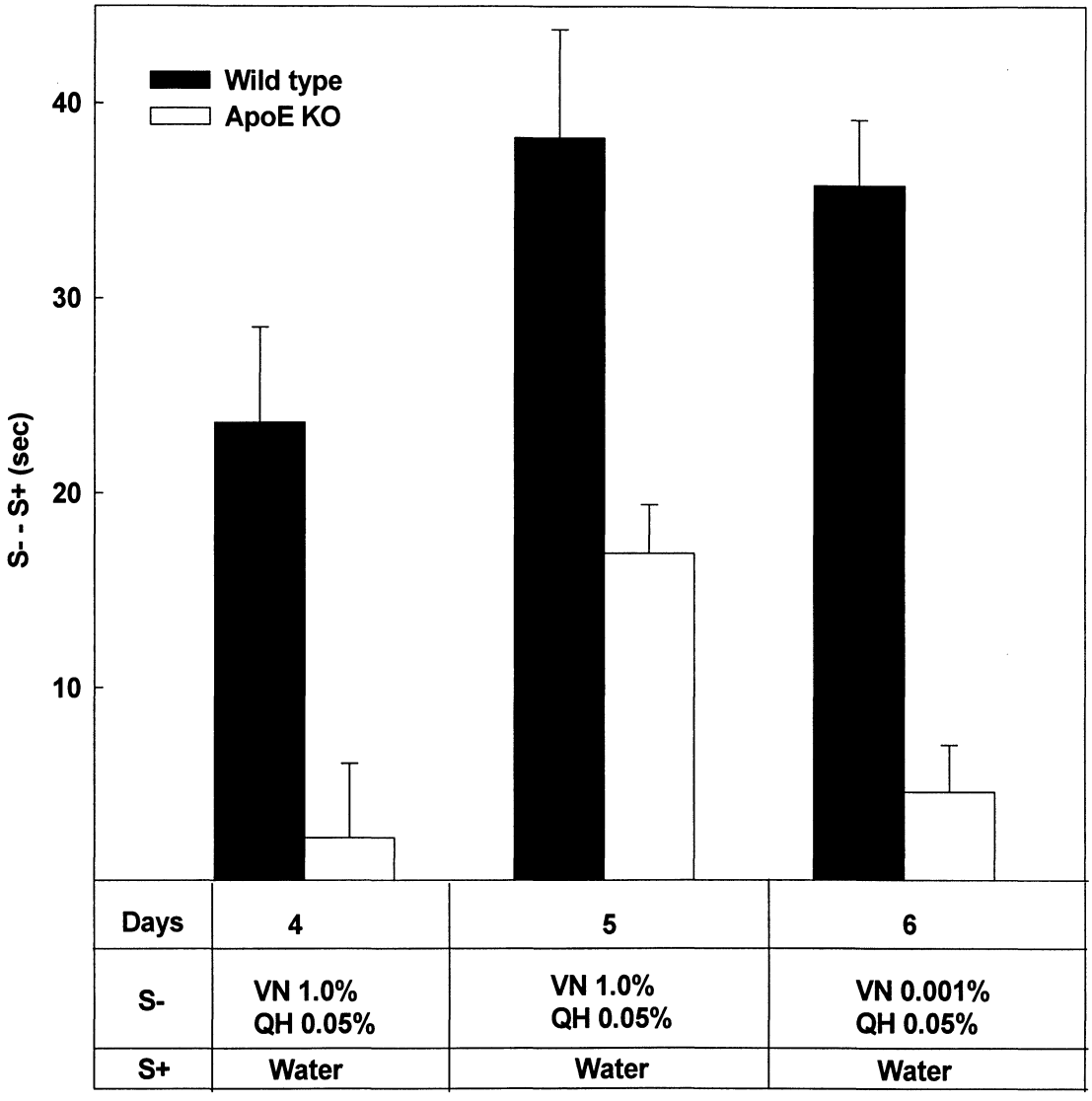


Figure 7. Wild type mouse OCTA latency on day 1 (S+)

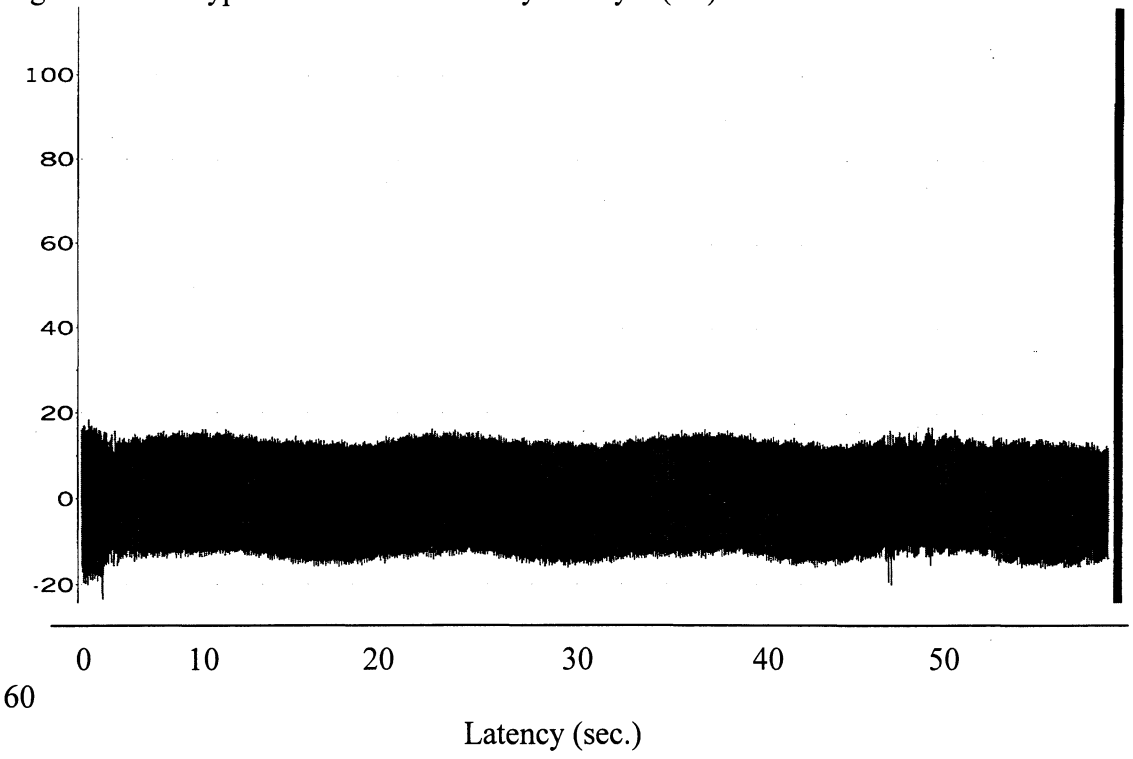


Figure 8. ApoE KO mouse OCTA latency on day 1 (S+)

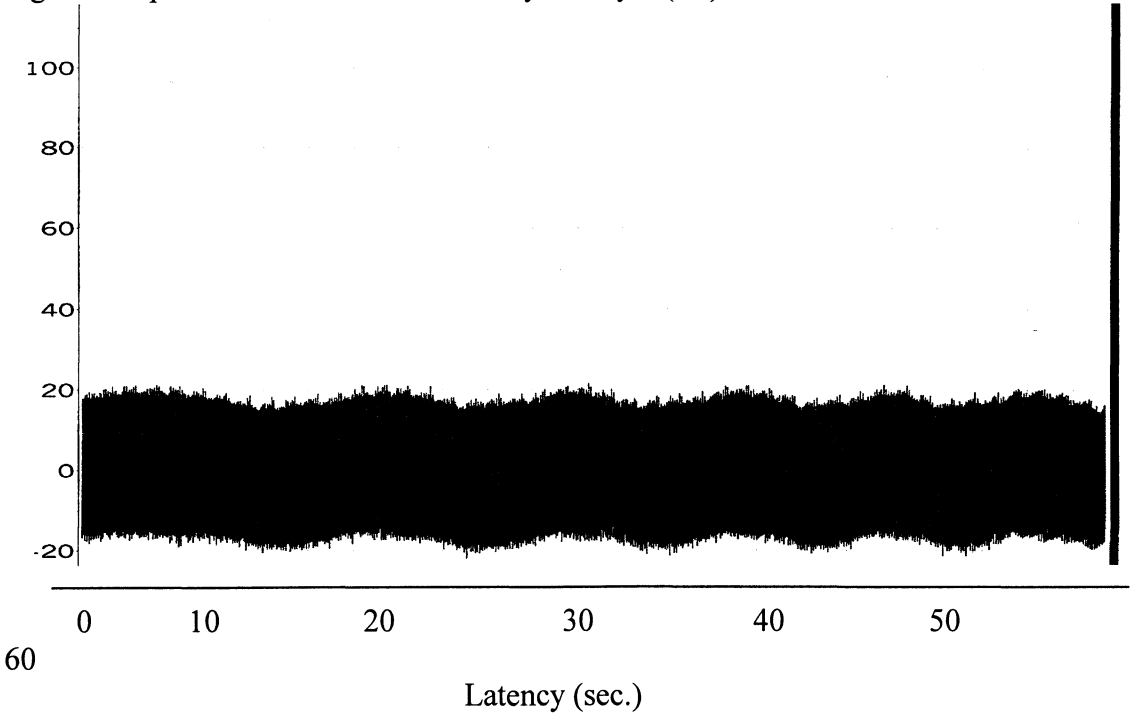


Figure 9. Wild type mouse OCTA latency on day 2 (S+)

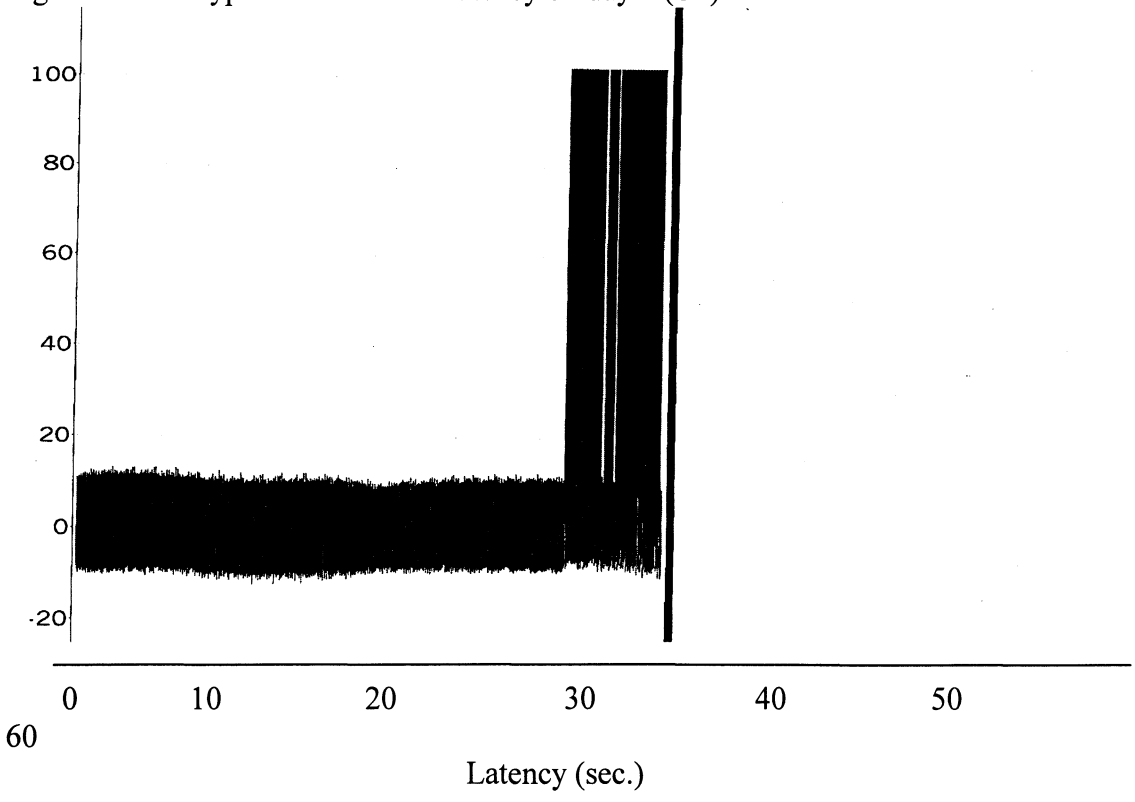
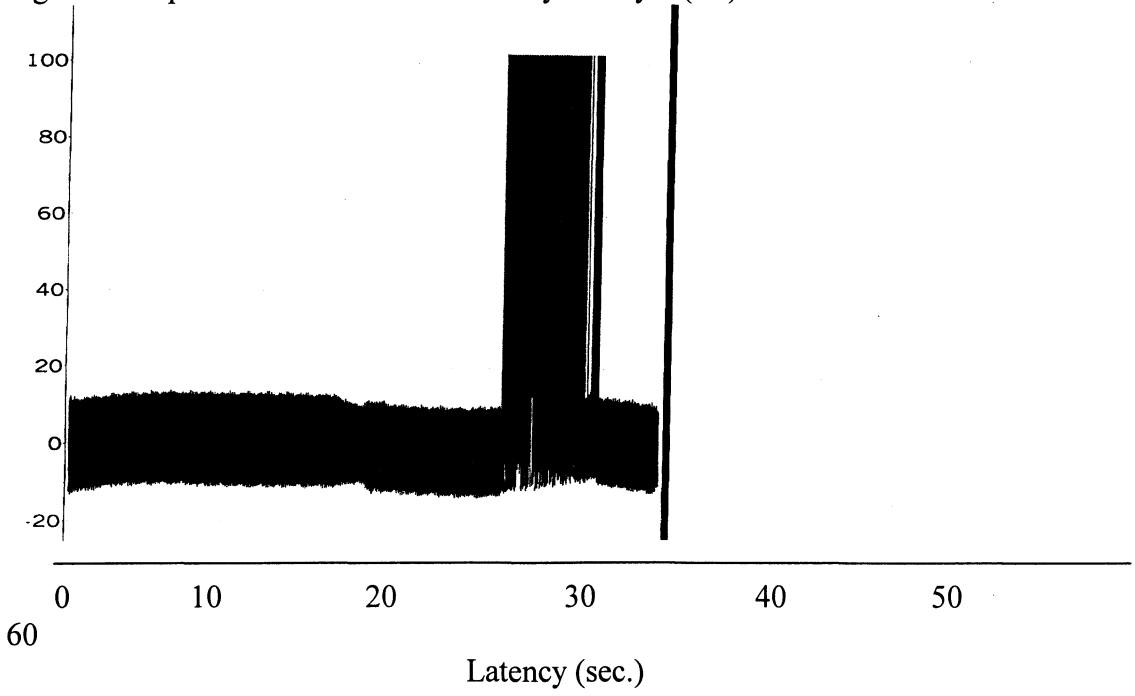


Figure 10. ApoE KO mouse OCTA latency on day 2 (S+)



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