Deficits in odor-guided behaviors in the transgenic 3xTg-AD female mouse model of Alzheimer's disease

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Abstract

Alzheimer’s disease (AD) is characterized by a number of alterations including those in cognition and olfaction. An early symptom of AD is decreased olfactory ability, which may affect odor-guided behaviors. To test this possibility we evaluated alterations in sexual incentive motivation, sexual olfactory preference, sexual olfactory discrimination, nursing-relevant olfactory preference and olfactory discrimination in female mice. We tested 3xTg-AD (a triple transgenic model, which is a “knock in” of PS1M146V, APPSwe, and tauP300L) and wild type (WT) female mice when receptive (estrous) and non-receptive (anestrous). Subjects were divided into three groups of different ages: (1) 4–5 months, (2) 10–11 months, and (3) 16–18 months. In the sexual incentive motivation task, the receptive 3xTg-AD females showed no preference for a sexually active male at any age studied, in contrast to the WT females. In the sexual olfactory preference test, the receptive WT females were able to identify sexually active male secretions at all ages, but the oldest (16–18 months old) 3xTg-AD females could not. In addition, the oldest 3xTg-AD females showed no preference for nursing-relevant odors in dam secretions and were unable to discriminate between cinnamon and strawberry odors, indicating olfactory alterations. Thus, the present study suggests that the olfactory deficits in this mouse model are associated with changes in sexual incentive motivation and discrimination of food-related odors.

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1. Introduction

Different animal models of Alzheimer’s disease (AD) have been engineered to examine amyloid-β (Aβ) and tau pathologies, and they support the hypothesis that a similar interaction occurs in human brain neurodegeneration (Lewis et al., 2001; Nelson et al., 2012). Among the lasting effects on cognition and behavior (Billings et al., 2007, 2005; LaFerla et al., 2007), it is well documented that olfactory alteration is one of the early symptoms in AD and provides an experimental tool to understand the mechanisms of synaptic dysfunction associated with the neuropathological progression (Wesson et al., 2010). The relation between olfactory perception and Aβ deposition in the olfactory system was
studied in the Tg2576 APP transgenic AD mouse model that over-expresses a mutated form of the human Aβ precursor protein (Wesson et al., 2010). It has also been demonstrated that short-term passive, anti-murine-Aβ immunization can restore olfactory behavior after Aβ deposition (Morales-Corraliza et al., 2013; Wesson et al., 2013). Moreover, in APP/PS1 transgenic mice, the locus coeruleus degeneration exacerbates olfactory deficits (Rey et al., 2012). The triple transgenic mouse model (3xTg-AD) harboring the human APPSwe, PS1M146V, and TauP301L gene mutations displays an age-dependent accumulation of both intracellular Aβ and tau proteins mainly within the cortex, hippocampus, and amygdala (Oddo et al., 2003a). Studies of odor memory in this 3xTg-AD model revealed severe olfactory deficits without gross changes in Aβ and tau immunoreactivity in the olfactory bulbs (Cassano et al., 2011).

It is well known that in mice, scents play key roles in the recognition of food odors as well as in sexual behavior, where they mediate recognition of a member of the opposite sex as a potential mate. The main and the accessory olfactory systems respond to pheromones involved in social communication (Baum and Bakker, 2013; Chamero et al., 2012; Korzan et al., 2013).

Female mice gain olfactory information through both the accessory and main system, and these cues are essential to stimulate attraction to an individual male’s scent (Hurst, 2009). The chemosensory system has important influences not only on sexual behaviors that are vital for reproductive success but also on the care of the offspring (Fraser and Shah, 2014; Keller and Lévy, 2012; Lévy and Keller, 2009). Mouse urine contains major urine proteins that bind to low-molecular-mass, volatile pheromones and protect them during their passage from the liver, and through the kidneys into the urine (Beynon and Hurst, 2003; Keller and Lévy, 2012). These volatile pheromones have profound effects on reproductive physiology and behavior (Tirindelli et al., 2009).

We designed this study to evaluate sexual incentive motivation, sexual olfactory preference, nursing-relevant olfactory preference and olfactory discrimination in transgenic (3xTg-AD) receptive and non-receptive females from 4 to 18 months of age. Females were chosen since the risk of AD is higher in females than in males.

2. Results

2.1. Sexual incentive motivation

The main objective of this behavioral test was to determine if 3xTg-AD female mice in estrous or anestrous showed a preference for a sexually active male or a sexually receptive female. The total distance traveled and velocity did not differ significantly between WT and 3xTg-AD mice at the ages studied (data not shown), indicating that these mice have no motor alterations. The sexual incentive motivation test showed that anestrous WT females at 4–5 months spent more time in the incentive zone of the female than that of the male ($\chi^2 = 17.66, P = 0.003$) (Fig. 1a). WT females in estrous, at all ages, spent significantly more time in the male incentive zone than in the female incentive zone (Fig. 1b) ($\chi^2 = 23.98, P = 0.0002$). Fig. 1c and d shows that 3xTg-AD females in anestrous and estrous at all ages studied (4–5, 10–11, and 16–18 months) showed no preference for either the male or the female (anestrous, $\chi^2 = 6.82, P = 0.23$ and estrous, $\chi^2 = 10.6, P = 0.59$).

2.2. Sexual olfactory preference

At 4–5 months of age, non-receptive female mice, both WT and 3xTg-AD, showed a significant preference ($\chi^2 = 23.34, P = 0.007$) for the estrous female secretions (EFS) compared to clean bedding but no preference for sexually active male secretions (SAMS). This preference for the EFS persisted at 16–18 months of age only in the transgenic mice ($\chi^2 = 19.68, P = 0.001$), as shown in Fig. 2a and e. At 4–5 months, both groups of mice in estrous showed a clear preference for SAMS ($\chi^2 = 26.64, P = 0.001$) over clean sawdust and EFS (Fig. 2b). At 10–11 months, WT females preferred SAMS to EFS and clean sawdust ($\chi^2 = 21.34, P = 0.007$), but 3xTg-AD females showed only a significant preference only for SAMS over clean sawdust (Fig. 2d). At 16–18 months of age WT females in estrous showed a significant preference for the SAMS sawdust ($\chi^2 = 25.31, P = 0.001$), while 3xTg-AD females in estrous showed no preference (Fig. 2f).

2.3. Nursing-relevant olfactory preference

The main objective in this behavioral test was to determine if 18-month-old female WT or 3xTg-AD mice show a preference for dam secretions (DS). This age was chosen because at this time the AD pathology is clearly established. The WT females showed a statistically significant increase ($\chi^2 = 19.43, P = 0.0002$) in the time spent with the DS compared with the clean sawdust, whereas 3xTg-AD females spent a similar amount of time smelling both types of bedding, as shown in Fig. 3.

2.4. Discrimination of food-related odors

At 4–5 months both groups (WT and 3xTg-AD) were able to discriminate cinnamon from strawberry ($\chi^2 = 0.1227, P = 0.002$), as shown in Fig. 4a. At 10–11 months of age, WT female mice significantly increased ($\chi^2 = 0.0854, P = 0.01$) the time spent sniffing a different odor, indicating that they were able to distinguish between new odors (saline from cinnamon, and cinnamon from strawberry), Fig. 4b. At 16–18 months of age they only discriminated between cinnamon and strawberry. On the other hand, 3xTg-AD mice were not able to discriminate between these odors at 10–11 or 16–18 months of age, Fig. 4c.

3. Discussion

The results of the present experiment indicate that 3xTg-AD female mice had a reduced sexual-incentive response to the sexually active male at all ages tested, which may be a consequence of diminished odor discrimination ability. This ability is sexually dimorphic and mostly of a motivational/sexual nature. Female WT mice respond more reliably than
male mice to low concentrations of volatile urinary odors from either sex (Pierman et al., 2006). Differences in olfactory sensitivity suggest that changes in gonadal hormones may affect olfactory capabilities in 3xTg-AD female mice. Behavioral data in WT female mice indicate that both the main and accessory olfactory systems participate in mate recognition (Baum and Bakker, 2013). Stimulation of the olfactory tubercle in WT mice also alters the odor-preference behavior as well as the c-Fos activity in neurons of motivational brain centers such as the forebrain, nucleus accumbens and lateral septum (Fitzgerald et al., 2014). On the other hand, it has been established that AD is accompanied by changes in mood associated with endogenously expressed APP in limbic, hypothalamic, and midbrain nuclei and with an increase in circulating cortisol levels, suggesting that regulation of the stress-responsive hypothalamic-pituitary-adrenal axis is disturbed (Guo et al., 2012). Thus, the alterations in the odor preference behavior may be associated with alterations in the motivational centers in this 3xTg-AD model.

We also found age-dependent differences in odor-preference behavior in the female WT group during the estrous stage as shown in Fig. 1b; these are novel findings since there are no previous reports considering odor-preference behavior during estrous across the lifespan in WT or in 3xTg-AD females (i.e., Morales-Corraliza et al., 2013; Wesson et al., 2010). Interestingly, urine from adult male mice contains high levels of pheromones which can regulate the reproductive state of females to match the prevailing social circumstances (Novotny and Soini, 2013), induce estrous and synchronize cyclicity in adult females (Brock and Bakker, 2011). These regulatory mechanisms may change with age in this model (3xTg-AD), as shown by the absence of significant behavioral differences, Fig. 1d.

The deficits may stem from Aβ deposited within the first synaptic processing stage of the olfactory system (Wesson et al., 2010). The ability of experimental animals to identify novel odors associated with food is an important tool used to identify alterations in olfactory receptors. In 3xTg-AD females, only young animals (4–5 months) were able to discriminate

Fig. 1 – Sexual incentive motivation in WT and 3xTg-AD female mice. WT and 3xTg-AD females were tested in anestrous (a and c) and estrous (b and d), respectively. Time spent in the female and male incentive zone was recorded and is expressed as Mean ± SEM (U-Mann Whitney test: *P < 0.05 and **P < 0.001).
between two food odors: cinnamon and strawberry; this discrimination was not found in the middle-aged and the oldest groups (10–18 months). Cassano et al. (2011) found severe deficits in odor-based memory in 3xTg-AD mice without Aβ and tau deposits in the olfactory bulbs, although marked immunostaining was present in the cortex and hippocampus, suggesting that impairment of olfactory-based information processing might arise from degenerative mechanisms that mostly affect higher cortical regions and limbic areas. Our preliminary histological examination of 4-, 10-, and 18-month-old females indicated progressive increases of Aβ and tau immunoreactivity in the olfactory area (anterior nucleus) as well as clear formation of plaques accompanied by immunohistochemical reactivity for glial fibrillary acidic protein (GFAP) (data not shown). A similar

Fig. 2 – Sexual olfactory preference for estrous female secretions (EFS) and sexually active male secretions (SAMS) in WT and 3xTg-AD female mice. Females of different ages were tested when they were in anestrous (a,c,e) and in estrous (b,d,f). Females were tested at 4–5 months old (mo) (a,b), 10–11 mo (c, d), and 16–18 mo (e and f). Data is expressed as Mean ± SEM (U-Mann Whitney test: *P < 0.05 and ** P < 0.001).
Effect is also observed in APPswePS1dE9 mice, in which plaque-associated reactive astrocytes and detectable levels of GFAP isoforms are observed (Kamphuis et al., 2012); these changes may affect synaptic connectivity within the olfactory area.

In the sexual incentive motivation test the experimental animals can see, hear, and smell the incentive animals but do not have physical contact with them because the stimulus animals are confined behind wire mesh openings. This method is similar to the one consistently used to measure sexual incentive motivation (Agmo, 2003; Agmo et al., 2004; Portillo and Paredes, 2003; Portillo et al., 2013), and it also provides new data showing that the receptive WT females of all ages spent significantly more time with the sexually active male. As we pointed out in the methods section, the stimulus female is a social incentive and is approached for social interaction, while the male is a sexual incentive and is approached both as a sexual partner and for social interaction (Agmo et al., 2004). In our study, anestrous WT females of 4–5 months were not sexually receptive and had no sexual experience; they spent more time with the social incentive (female). At older ages (10–11, 16–18 months) anestrous females, as expected, spent the same time with the sexual and social incentives. At younger ages females are probably more willing to socialize than at older ages. In contrast, the 3xTg-AD females exhibited no preference for the stimulus male at any age. This could be due to the lower level of estrogen receptors reported in 3xTg-AD females (Caruso et al., 2013).

Previous studies have shown that olfactory cues in rats are crucial for sexual partner preference (Agmo, 2003; Portillo et al., 2006). These cues include urine, feces, and vaginal discharges that can be collected and presented to the subjects. Sexually naive females show a clear preference for odors from sexually experienced males (Osada et al., 2008). In the present experiment, WT and young 3xTg-AD mice showed a clear preference for SAMS, which are attractive to female mice in estrous, probably due to their high pheromone level.

In addition, we performed a behavioral task in which the oldest WT and 3xTg-AD females (18 months of age) were in contact with DS; the results revealed that the oldest WT females were able to discriminate between clean and DS sawdust, as opposed to the oldest 3xTg-AD females who spent the same amount of time in both types of sawdust (clean and DS), indicating a lack of preference for DS odor. Since these WT and 3xTg-AD females had nursery experience (all were multiparous with more than 4 deliveries), this test evaluates their retrograde olfactory memory, providing information about the severity of the behavioral changes because retrograde memory is affected by AD later than anterograde memory (Calkins and Reddy, 2011). Thus, the behavioral deficit in old 3xTg-AD females was not associated with a lack
of maternal sensory experience but rather could be ascribed to the pathology. However, since there are few studies describing estrogen levels in this transgenic model (Carroll et al., 2010, 2007) it will be necessary to determine if they varied with the pathology. It also needs to be determined if there is correlation between tau pathology and the soluble amyloidogenic material in the olfactory system.

In 3xTg-AD mice, altered olfaction and impaired cognitive flexibility in the first six months of life have been reported (Marchese et al., 2014), suggesting that manifestations similar to mild cognitive impairment occur before general learning/memory impairments emerge at older ages. In our study a deficiency in the discrimination between food-related odors was found from 10 months of age, and this deficiency increased progressively until the age (16–18 months) when the pathology was installed.

In addition, 3xTg-AD female animals from 10 to 18 months of age were unable to discriminate among food-related odors, suggesting an olfactory deficit that may be related to the amyloid-β deposits in the olfactory areas. Thus, we found that female 3xTg-AD mice have clear deficiencies in their olfactory preference and discrimination, which could be due to an inability to recognize sexually relevant odors. Measurements of Aβ deposition in the olfactory bulb as well as in the olfactory epithelium will be necessary to further evaluate if the behavioral deficits are associated with histological alterations.

4. Experimental procedures

4.1. Subjects

The experiments were carried out in accordance with the “Reglamento de la Ley General de Salud en Materia de Investigación para la Salud” of the Mexican Health Ministry which follows NIH guidelines and were approved by the local animal care committee. All animals were maintained with a 12-h/12-h inverted light/dark cycle (lights off at 9:00 h) at 22–23 °C and 40–50% humidity with free access to food (standard Purina Chow, formula 5001) and purified water. For breeding, colonies of 3xTg-AD (homozygous mutant APPsw, PS1M146V, and Taut301L) mice and their background strain, wild type (WT, C57BL/6/129S2/C2) mice from the Jackson Laboratory (Bar Harbor, ME) were used. Transgenic mice were identified by tail DNA isolation using established procedures (Oddo et al., 2003b). Two females and one male per cage (total of 20 reproductive units) were housed in polycarbonate cages (12 × 12 × 25 cm³) maintained in ventilated racks during mating and gestational periods. Pups were weaned at 30 days, and then housed separated by sex: 3 males or 3 females per cage until the ages studied.

A total of 144 female mice (WT and 3xTg-AD) were used for two behavioral tests at three ages (4–5 months old, 10–11 months old, and 16–18 months old) as well as two conditions (receptive and non-receptive). Eight animals per group were tested for sexual incentive motivation (interacting with a sexually active male or a sexually receptive female) and olfactory sexual olfactory preference (time investigating the bedding exposed to sexually active male or sexually receptive female secretions). After every test, female WT (129/C57BL/6) and transgenic (3xTg-AD), receptive (estrous) and non-receptive (anestrous) mice were examined to determine the stage of the estrous cycle they were in. A vaginal smear was collected after the task with a vaginal swab using an inoculating loop. The inoculator tips were wetted in 50% methylene blue (dissolved in saline) and inserted into the vagina, gently scratching the vaginal wall. The swab was placed on a glass slide and overlaid with a coverslip. Samples were immediately analyzed at 20 × magnification under bright field illumination. The estrous stage was determined by the presence of cornified and nucleated epithelial cells (Byers et al., 2012; Felicio et al., 1984).

Other 16- and 18-month-old females (WT and 3xTg-AD) were tested for nursing-relevant olfactory behavior (time investigating bedding impregnated with dam secretions). Additionally, 48 females (WT and 3xTg-AD) at the same three ages were tested for olfactory discrimination (time investigating two food-related odors: strawberry and cinnamon).

4.2. Behavioral testing

4.2.1. Sexual incentive motivation

In ovariectomized females, hormone replacement with estradiol and progesterone induces sexual receptivity, and females spend more time in the intact male incentive zone as opposed to the castrated male incentive zone. On the other hand, ovariectomized female mice without hormone replacement do not display sexual behavior, and they spend the same amount of time in the incentive zone of the castrated and intact males (Agmo et al., 2008). The test was performed in accordance with the procedure described by Agmo (2003) and adapted to mice (Portillo et al., 2013). The test was carried out in a rectangular acrylic arena (100 × 50 cm²) with one opening covered with wire mesh at floor level on each long wall (100 cm long). A small acrylic compartment (25 × 25 × 15 cm³) was assembled at each opening. For the test, a sexually receptive female was placed in one compartment, and a sexually active male was placed in the other compartment. Outside the mesh wall a zone (20 × 30 cm³), called the incentive zone, was designated. In this test the stimulus male is considered the sexual incentive because, if given the opportunity, the subjects would probably mate. The stimulus female is the social incentive, because the females will not mate with same sex mice (Agmo et al., 2004).

This arrangement allows the experimental animal to hear, smell, and see the stimulus mice, but physical contact is prevented. Before the test, WT (n=8) and 3xTg-AD (n=8) females were habituated to the arena without the stimulus animals in three 10-min sessions; during this pretest the total distance and velocity of exploration were recorded in order to detect if the females had general motor alterations. During the test, each experimental female was gently placed in the center of the arena, and a Panasonic SD II (super dynamic) camera connected to a computer registered the time that the experimental female spent in the incentive zone of each stimulus animal. The behavioral data was tracked and analyzed using Noldus EthoVision 3.0 video tracking software. This test was repeated one week later, when the location of the stimulus animals was reversed.
4.2.2. Sexual olfactory preference

The olfactory preference task was done in a transparent acrylic cage (44 × 23 × 21 cm³) following the procedure described by Portillo and Paredes (2003). Three small plastic containers (8.5 × 8.5 × 2.5 cm³) containing either clean bedding (clean sawdust), estrous bedding (sawdust collected from the cage of five sexually receptive females), or male bedding (sawdust collected from the cages of five single-housed males) were randomly placed inside the cage. The bedding had been in contact with the corresponding group for 6 h before it was used. The time spent smelling the bedding in each container was registered. The test lasted 10 min and was repeated 48 h later for both experimental groups (WT and 3xTg-AD).

4.2.3. Nursing-relevant olfactory preference

In order to determine if 3xTg-AD mice had alterations in the preference for nursing-relevant odors, 18-month old females were placed in a transparent acrylic cage (44 × 23 × 21 cm³) in which two small plastic containers (8.5 × 8.5 × 2.5 cm³) were randomly located. Each container was filled with 5 g of either clean sawdust or sawdust collected from the cages of five, single-housed lactating females with their pups. In this latter case, the sawdust had been exposed for 6 h to the lactating females and their pups, and the bedding of all five cages was mixed. The two containers were randomly placed inside a transparent acrylic cage (44 × 23 × 21 cm³). We registered the time spent by 18-month-old females on top of each plastic container with the different types of sawdust for each WT (n=8) and 3xTg-AD (n=8) mouse. The test lasted 10 min and was repeated 48 h later.

4.2.4. Discrimination of food-related odors

This test is based on the habituation–dishabituation paradigm described by Gregg and Thiessen (1981) and was performed in an acrylic cage (27 × 16 × 12 cm³) covered on top with a wire mesh over the cage grid. A filter paper (0.5 × 0.5 cm²) attached to a plastic container (4.5 × 4.5 × 1 cm³) was impregnated with 10 μl of either saline or a food-related volatile odor (strawberry or cinnamon). These two odors were selected from among nine different odors (i.e. orange, lemon, tangerine, strawberry, banana, cinnamon, lavender, eucalyptus, and mint) based on preliminary novel odor recognition trials with other mice (data not shown). During the test, the filter paper with the selected odor was placed on the wire mesh. The protocol was as follows: two trials (trial duration of 2 min and inter-trial interval of 1 min) of saline followed by three trials of one of the odor stimuli, followed by presentations of the second odor. Saline was always presented first, followed by the three presentations of the cinnamon stimulus and later the 3 presentations of the strawberry stimulus. The test was performed in duplicate (48 h between sessions), changing the order of the stimulus presentation. The time spent smelling the filter paper with the stimulus was registered in all groups.

4.3. Data collection and statistics

The data were statistically evaluated using StatView V5.0.1 and GB-STAT V10 statistical software. Data from the sexual incentive motivation (including distance, velocity, and time measurements), sexual olfactory preference and nursing-relevant olfactory preference were not normally distributed, and they were subjected to a nonparametric analysis using the Kruskall–Wallis test followed by the U Mann–Whitney test. Data from discrimination of food-related odors was assessed with Friedman’s ANOVA, and the post hoc comparisons were performed with the U Mann–Whitney test. Effects with a P value <0.05 were considered to be statistically significant.

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