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Spatial Disorientation Under Dark Conditions Across Development in an Alzheimer's Disease Mouse Model

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Abstract—Alzheimer's disease (AD) is associated with hippocampal neuropathology and cognitive impairments, including wandering behavior or becoming lost in a familiar environment. Wandering behavior is severe and manifests early in life for people with specific genetic mutations. Genetic mouse models of AD have been developed to characterize the onset and progression of behavioral deficits that represent human behaviors, such as wandering, to test the efficacy of therapeutics. It is not clear if current assessments of mouse models capture the onset of AD or a snapshot of its progression. Sequential analysis of open field behavior provides a robust, quick test to dissociate navigation cues that contribute to spatial disorientation, a feature of wandering. Despite potential utility in evaluating this feature of AD, little work has been reported using animal models of dementia in this task. Thus, we examined the use of different sources of information to maintain spatial orientation at two prodromal ages in female transgenic CRND8 AD (n = 17) and Control mice (n = 16). These mice exhibit amyloid plaques, a hallmark neuropathological feature of AD, that are associated with cognitive dysfunction at ~three months of age. Spatial disorientation was observed at two months and more severely at four months under dark conditions, but performance was spared when visual environmental cues were available. This study provides documentation of impaired self-movement cue processing in AD mice, establishing the dark open field as a behavioral tool to characterize spatial disorientation associated with AD. These findings may accelerate future assessments of novel therapeutic interventions for neurological disorders.

Key words: Alzheimer's disease, TgCRND8 mice, spatial disorientation, self-movement cues, dark open field, acetylcholinesterase.

INTRODUCTION

Alzheimer's disease (AD) is characterized by a progressive increase in neuropathology and cognitive impairments (Glenner and Wong, 1984; Goate et al., 1991; Kandel et al., 2000; Mesulam, 2000; Jalbert et al., 2008). Plaques and neurofibrillary tangles are the two main neuropathological features of AD with plaques beginning to develop roughly 20 years before AD is diagnosable currently (Anderton and Brion, 1991; Bateman et al., 2012; Pospich and Raunser, 2017). Neuropathology typically begins within the basal forebrain and, as it accumulates throughout the brain, outward mild cognitive impairment gradually develops. Eventually, severe deficits in memory (i.e., word recall, visuospatial skills) and other domains emerge, including spatial orientation and executive function. The degeneration of cholinergic neurons in the basal forebrain has been posited as contributing to these cognitive deficits early in AD. This led to the development of "the cholinergic hypothesis" which has guided AD research for over the past 50 years (Craig et al., 2011). Pharmaceutical agents developed to act on the cholinergic system have shown limited therapeutic effects in the treatment of AD (for review see Hampel et al., 2018), perhaps because treatment begins too late in the disease progression. Behavioral assessments and diagnosis typically occur once outward deficits have manifested when AD has already begun to progress. This timeframe may be too late for the implementation of effective treatments.

A variety of genetic mouse models, based on human mutations associated with AD, have been developed to investigate the onset and progression of AD-related neuropathology with the goal of finding a treatment to slow the progression of the disease (Mineur et al., 2005). Mutations in amyloid precursor protein (APP) and presenilins1 and 2 lead to neuropathological changes, such as cell death and atrophy of the brain, in both mouse models and patients with AD (Chapman et al., 1999; Palop et al., 2003; Bertram et al., 2010; Elder et al.,

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Abbreviations: Alzheimer's disease, AD; Transgenic, Tg; Amyloid protein precursor, APP; Complementary deoxyribonucleic acid, cDNA.

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2010; Luccarini et al., 2012). The transgenic (Tg) CRND8 mouse model expresses human APP containing several mutations associated with a genetic form of early onset AD. In this model, amyloid beta plaques begin to develop early in life, by three months, providing an accelerated AD mouse model that has similar features as familial AD. TgCRND8 mice and patients with AD also experience gradual cognitive impairments which manifest around the same time that plaques accumulate in the brain, making this an ideal model to begin elucidating the effects of early pathology on wandering behavior.

Some of the earliest reported impairments in AD include memory, with patients often experiencing spatial disorientation, accompanied by wandering behavior (Logsdon et al., 1998). Wandering behavior has been attributed to becoming lost in familiar environments, such as trying to find one's way home from a store. This poses a severe risk to patients with AD as those that are not found within the first 12 hours have less than a 20% survival rate (Koester and Stooksbury, 2015). Mouse models of AD have been found to recapitulate some of the locomotor behaviors and spatial orientation deficits seen in humans with the disease, though most of this work has focused on late effects of the disease after overt behavioral and pathological abnormalities have emerged. Many failed drug development attempts for AD suggest that treating the disease after such overt onset may be futile. Therefore, it is crucial to develop sensitive assessments that can identify deficits earlier in disease progression (i.e., during the prodromal stage) to create a larger timeframe for interventions and to alert individuals of potential behavioral threats, such as wandering.

Disruptions in performance on spatial tasks are observed in genetic mouse models of AD (Janus et al., 2000; Chishti et al., 2001; Lovasic et al., 2005; Geekiyanage et al., 2013) and in patients with AD. Many of the tasks used to assess spatial function only evaluate one aspect of spatial orientation, such as the use of environmental cues to guide navigation. Rodents hierarchically use environmental (visual or olfactory) and self-movement (vestibular or proprioceptive) cues to guide open field behavior depending on the availability of resources in the environment (Maaswinkel and Whishaw, 1999). For example, animals placed in an open environment will organize their behavior in a series of stops and progressions focused around a home base (Eilam and Golani, 1989; Golani et al., 1993; Tchernichovski and Golani, 1995; Drai and Golani, 2001; Wallace et al., 2002, 2006; Wallace, 2017). Although environmental cues can anchor the organization of this behavior, similar organization is observed under conditions without access to visual or olfactory cues (Hines and Whishaw, 2005). The organization of open field behavior under conditions with varied access to environmental cues provides an assessment of spatial disorientation associated with neurological disorders. While previous work has primarily used spatial tasks that provide access to multiple sources of information, behavioral assessments that can dissociate environmental and self-movement cue processing may provide increased sensitivity to detect developmental changes in rodent models of AD.

The role of basal forebrain cholinergic system in the progressive cognitive impairment associated with AD has been debated for decades. Early work observed that degeneration of cholinergic basal forebrain structures was associated with the cognitive decline in patients with AD (Perry et al., 1978; Bartus et al., 1982). In contrast, the development of techniques to selectively deafferent hippocampal cholinergic projections in rats (Wiley et al., 1991) failed to produce impairments on traditional mnemonic spatial tasks (Baxter and Gallagher, 1996; Jonasson et al., 2004; Vuckovich et al., 2004; Frielingsdorf et al., 2006). However, assessments that dissociate the source of information used to maintain spatial orientation have demonstrated a role for the septohippocampal cholinergic system in processing self-movement cues (Martin and Wallace, 2007). These observations parallel work showing a progressive impairment in processing self-movement cues (optic flow) that is predictive of wandering behavior in patients with AD (Tetewsky and Duffy, 1999).

It is currently unknown whether a mouse model of AD will exhibit spatial disorientation that is selective to self-movement cue processing during the prodromal period, before to the onset of amyloid beta plaques. Further, whether such behavioral deficits are associated with acetylcholinesterase levels need to be examined. Thus, the current study investigates spatial orientation and acetylcholinesterase in the TgCRND8 mouse model of AD at two and four months of age.

EXPERIMENTAL PROCEDURES

Subjects

Male TgCRND8 mice and female non-transgenic mice were bred to generate the mice used in this study. Breeders had low numbers of offspring per litter and because male TgCRND8 mice were used for breeding to continue the line and increase population diversity, only female TgCRND8 and non-transgenic littermates were assessed in these experiments. TgCRND8 mice, referred to AD mice in this study, harbor the human APP695 complementary deoxyribonucleic acid (DNA) transgene with the Swedish (K670M, N671L) and Indiana (V717F) mutations (Tg+/−) [19–20]. Age-matched non-transgenic (Tg−/−) mice, referred to as control mice in this study, based on the same background strain, were used as controls. No more than three mice came from the same litter. Mice were group housed in consistent vivarium temperatures (20–21 °C) on a 12-hour light/dark cycle and provided food and water *ad libidum*. The Institutional Animal Care and Use Committee at Northern Illinois University approved all procedures described in this experiment and all guidelines set by the Office of Laboratory Animal Welfare were followed.

Genotyping

DNA was isolated from mouse ear punches at one-month of age and from tail snips during histological analysis. Genotyping of mice for the presence of the human APP

gene was carried out as previously described (Hinrich et al., 2016) to determine AD and control mice.

Apparatus

The open field apparatus was a circular white table (122 cm) without walls located 1.3 m above the floor in a room with many cues, including posters, a cabinet, and a door. A video camera was located directly above the center of the apparatus to record each trail. Infrared lights illuminated the testing room and night vision goggles were used to handle mice during all open field procedures under dark conditions. At the beginning and end of each trial, a mouse was placed in a transport cage, covered with a towel, and led on a circuitous path to and from the testing room to reduce the possibility of using the colony room as an anchor for spatial representations. The table was thoroughly cleaned and prepared for the subsequent trial between each mouse.

Procedures

Thirty-four female mice received one 40-minute open field session under completely dark conditions at two months of age followed one week later by another 40-minute open field session under light conditions. Occasionally, a mouse fell off the table during an open field session, in which case, it was placed back on the table for the remaining duration of the open field session to collect 40 minutes total of open field behavior. Mice were tested at approximately the same time each testing session during the light portion of the light/dark cycle. Video recordings of open field behavior were saved and used for subsequent motion capture analysis of stops, progressions, and stop cluster analysis.

A subset of mice (n = 16) was perfused after the two-month light open field session and used to analyze acetylcholinesterase in the hippocampus and cortex and to assess optical densities. The remaining mice (n = 18) were left undisturbed until four months of age. Then, these mice received another 40-minute dark open field session followed one week later by a 40-minute light session. After the light session, mice were perfused, and brains were collected for histological analysis of acetylcholinesterase optical densities in the hippocampus and cortex.

Histological analyses

Mice were intracardially perfused at either nine (Control: n = 8, AD: n = 8) or 17 weeks old (Control: n = 9, AD: n = 8) immediately following the light open field session. An 8% phosphate buffered saline solution was first used to clear the brain of blood followed by 4% paraformaldehyde to fix the brain for histological analysis. Brains were left in paraformaldehyde for 24 hours after perfusions and then transferred to a 30% sucrose mixture for cryoprotection. Once the brains had sunk, indicating cryoprotection, a cryostat was used to cut the tissue to 40 μm slices. Slices were directly mounted to pre-double subbed gelatin slides and left to dry overnight. Then, slides were stained for acetylcholinesterase as described previously (Silver, 1974). Briefly, Tetraisopropyl pyrophophoramide (6.24 mg) was placed in 200 ml of distilled water and stirred until dissolved. Once the Tetraisopropyl pyrophophoramide had dissolved, slides that were placed horizontally in a tray were carefully put into this solution and stirred lightly on a belly dancer for 30 minutes. During this 30-minute bath, the next reaction mixture was prepared by adding the following chemicals chronologically to 200 ml of distilled water while stirring lightly: 5,720 mg Trizma maleate buffer, 2,040 mg Trizma base, 100 mg Acetlthiocholine iodide, 294 mg Sodium citrate, 150 mg Cupric citrate, and 32.8 mg Potassium hexacyanogerrate (III). The slide tray was removed from the Tetraisopropyl pyrophophoramide solution and directly and gently submerged twice in distilled water. The slide tray was placed in the reaction mixture and stirred lightly on a belly dancer for four hours. Then the slide tray was submerged twice in distilled water, laid flat to dry, and cover slipped the following day. After the slides dried, microscopic photos were captured, and ImageJ was used to calculate optical densities of cortical (dysgranular, granular, motor; Fig. 1A) and hippocampal (cornu ammonis 1, cornu ammonis 3, dentate gyrus; Fig. 1B) areas. Three 20X20 boxes were used to sample optical densities in each subregion which were then averaged across the hippocampus and cortex, respectively.

Data analyses

The Noldus Ethovision XT 13 motion capture system was used to track movement and conduct subsequent detailed analyses of 16-minute segments of open field behavior under dark and light conditions. Previous work has established that approximately 20 minutes of movement in the open field provides enough movement to evaluate performance metrics and account for potential falls (Blankenship et al., 2017; Donaldson et al., 2018; Banovetz et al., 2021; Osterlund Oltmanns et al., 2021; Schaeffer et al., 2022; Osterlund Oltmanns et al., 2022). Either the first consecutive 16 minutes were analyzed, or if a fall(s) occurred, then the first consecutive eight minutes of data were chosen along with a second consecutive eight minutes of data (i.e., where no falls occurred). Mice were given two minutes to acclimate to the environment once first placed on the table and after any falls, before any further data was acquired. If a mouse fell off the table during the open field session, this portion of the video was excluded from the analysis of movement organization. Reduction in the amount of locomotion across an open field session may reflect an increase in familiarity within environmental cues. In contrast, progressive loss of movement organization in the open field may reflect spatial disorientation. Therefore, the between sessions analysis provides further insight to group differences at each age.

Motion capture software was used to generate x-y coordinates to evaluate the kinematic organization of mouse movement at five frames per second. The x-y data was sorted into progressions (movement > 3 cm/s for at least two frames) and stops (movement < 2.99 c m/s for a minimum of two frames) to evaluate several

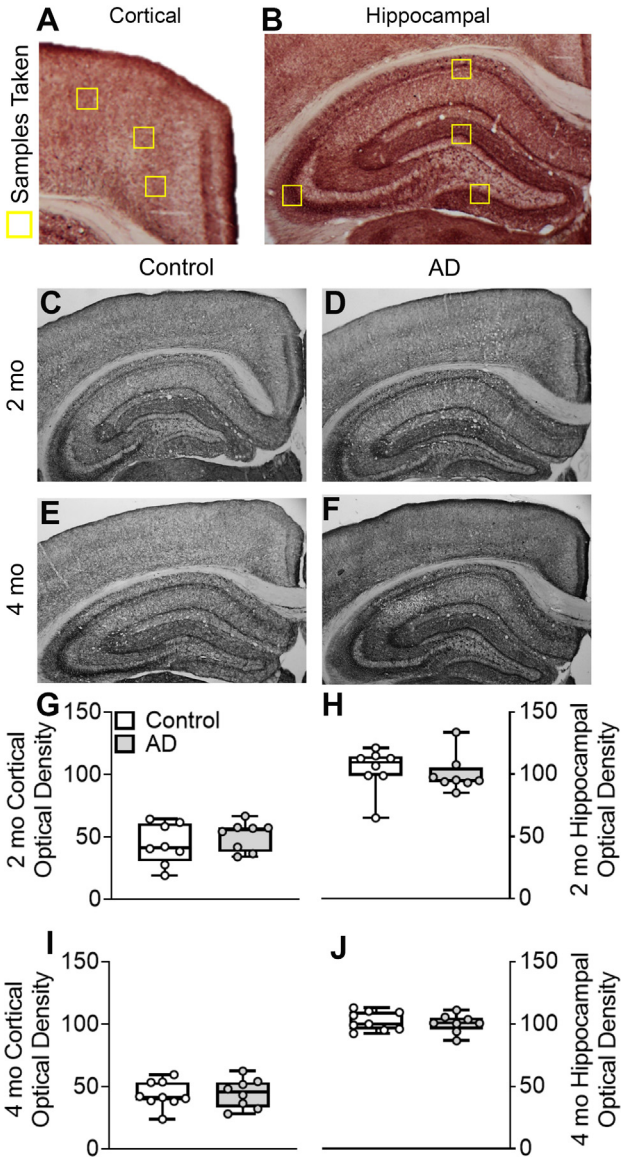

Fig. 1. Cortical **(A)** and hippocampal **(B)** microphotographs are shown with each sampled region boxed in yellow. Representative microphotographs are displayed for a control and AD mouse at two **(C, D)** and four **(E, F)** months of age. Acetylcholinesterase optical densities were similar at two **(G, H)** and four months **(I, J)** in the cortex and the hippocampus between control and AD mice. Box and whisker plots are shown with the bar extending from the 25th to 75th percentiles, and the line within the bar representing the group average. The whiskers go down to the smallest value and up to the largest value for each group, and individual data points are shown on each box plots.

measures of performance, such as general features of movement organization, and specific progression and stop characteristics. All measures were averaged across two eight-minute samples except for the progression class analyses that were averaged across each class (i.e., long, medium, short).

Stop characteristics

Stopping behavior can be indicative of spatial disorientation in several ways. Stops were characterized by change in heading, stop clustering within samples (first-order r) referred to as stop consistency, and stop clustering between samples (second-order r) referred to as home base stability during the open field session. Directional change in heading occurs during a stop between two progressions. Change in heading was calculated as the supplementary angle to the angle subtended by (1) the previous progression peak speed location, (2) the average stop location, and (3) the subsequent progression peak speed location. This measure ranges from 0° (continuing straight) to 180° (reversal of path). Typically, mice exhibit changes in heading around 60° in the open field, while larger changes in heading are associated with spatial disorientation (Banovetz et al., 2021).

Stop clustering was assessed using circular statistics (Batschelet, 1981). First, stop consistency was calculated from all theta stop values within (eight-minute) samples. Values closer to one denote greater stop consistency while values closer to zero represent less consistent stops. Usher mice that harbor a harmonin genetic mutation (Donaldson et al., 2018) and mice with bilateral sodium arsanilate vestibular lesions exhibit circling behavior which produced higher within-sample stop consistency relative to controls (Banovetz et al., 2021). Second, home base establishment was evaluated across open field segments. Strong home base establishments yield values closer to one and weak home base establishments generate values closer to zero. *Tilted* mice which lack otoconia show a drifty home base across time during a dark open field session (Blankenship et al., 2017).

General movement characteristics

General features of movement organization included falls off the table, average total distance traveled (cm), average total stop time (s), and the total percent of stops that occurred on the table periphery, providing information about general locomotion and anxiety-like behavior. Falls off the table were a total count of all falls that occurred during the 40-minute open field session. Average total distance was calculated as the average total distance traveled across each eight-minute sample. Progression peak speed (cm/s) was derived from the x-y data and associated time. Average total stop time was calculated as the average of all stopping behavior (movement < 2.99 cm/s for a minimum of two frames) across each eight-minute sample. Stops on the periphery were calculated by summing the percent of the total stops that occurred on the outer 10% of the table periphery.

Progression characteristics

Specific progression measures (> 10 cm to rid of small jitter/tuning movements) were assessed across the open field session. Then, progressions were further classified as long, medium, and short to provide a detailed analysis of movement during different gears (Drai et al., 2000; Zott et al., 2018; Banovetz et al., 2021; Osterlund Oltmanns et al., 2021). In addition to distance traveled and peak speed, a measure sensitive to spatial disorien-

tation, path circuity, was evaluated under dark and light conditions for overall progressions and for long, medium, and short progressions. Path circuity is a measure of progression directness (Euclidean distance of path/actual path distance) that ranges from one (straight path) to zero (highly circuitous path). Typically, long progressions are more circuitous than short progressions. Paths typically become more circuitous in rodent models of vestibular dysfunction that exhibit spatial deficits in the dark open field (Donaldson et al., 2018; Banovetz et al., 2021).

Statistical analyses

Independent samples t-tests were used to evaluate group differences in histology with Cohen's *d* (*d*) reported for the effect size. Repeated measures ANOVAs were used to investigate changes in both general and specific performance measures across the first and second halves of the open field session (i.e., two eight-minute samples), between groups, and Group by Sample interactions. Gear analyses across progression class (i.e., long, medium, short) were also evaluated using repeated measures ANOVAs. Partial eta squared (η^2_p) was reported for measures related to the gear analysis. Tukey HSD was used for post-hoc analyses with Cohen's *d* as a measure of effect size. Jasp 0.12.2 statistical software (University of Amsterdam) was used for all statistical analyses. The mean and standard error of the mean are shown in graphs.

RESULTS

Acetylcholinesterase

Acetylcholinesterase optical densities were assessed in the cortex and hippocampus of two and four-month-old mice (Table 1). Representative micrographic photos are shown for one control (Fig. 1C) and one AD (Fig. 1D) mouse at two months of age. At two months of age, acetylcholinesterase failed to differ between groups in the cortex [$t_{14} = 0.931$, $p = 0.368$, $d = 0.465$] and hippocampus [$t_{14} = -0.446$, $p = 0.663$, $d = -0.223$] (Fig. 1G).

Representative micrographic photos are also shown for a control (Fig. 1E) and an AD (Fig. 1F) mouse at four months of age. Four-month-old mice also failed to express different levels of acetylcholinesterase in the cortex [$t_{15} = 0.197$, $p = 0.846$, $d = 0.096$] (Fig. 1I) and hippocampus [$t_{15} = -0.590$, $p = 0.564$, $d = -0.287$] (Fig. 1J). All mice exhibited similar levels of acetylcholinesterase optical densities within the hippocampus and cortex at two and four months of age.

Open field behavior at two months of age

Dark conditions. At two months of age, during the dark session, falls off the table were infrequent. Control mice fell off the table an average of 0.294 (min = 0; max = 2) times, and AD mice fell off the table an average of 0.563 (min = 0; min = 2) times. Groups did not significantly differ in falls at two months under dark conditions [$t_{31} = 1.169$, $p = 0.251$, $d = 0.407$].

Mice organize open field behavior under dark conditions into progressions (Fig. 2A, B). The repeated measures ANOVA conducted on total distance revealed a significant effect of group and sample; however, the Group by Sample interaction was not significant (Table 1). Group differences were observed in the total distance travelled during each sample (Fig. 2C) with AD mice traveling significantly further distances relative to control mice. A significant decrease in distance travelled was observed across samples.

Mice exhibited stops of varied duration throughout the environment under dark conditions (Fig. 3A, B). The repeated measures ANOVA conducted on total stop time revealed a significant effect of sample; however, neither the effect of group nor Group by Sample interaction were significant (Table 1). Although groups did not differ in time spent stopping (Fig. 3C), there was a significant effect of Sample with all mice spending more time stopped in the second half of the session relative to the first half.

Stops usually occur near the edge of the table. The percent of stops that occurred along the outer most 10% of the table was found to be 68% for control and 76% for AD mice at two months under dark conditions. No differences were observed between groups in the percentage of stops that occurred around the periphery of the table [$t_{31} = 0.933$, $p = 0.358$, $d = 0.325$].

Most of the change in heading during open field behavior under dark conditions occurred during stops (Fig. 4A, B). The repeated measures ANOVA conducted on change in heading failed to reveal a significant effect of group, sample, and Group by Sample interaction (Table 1). Groups did not differ in change in heading (Fig. 4C), and no differences were observed across samples.

The location and duration or stop clustering within the open field varied under dark conditions. The parameter of concentration is a measure of stop clustering density within a direction (Fig. 5A, B). The repeated measures ANOVA conducted on parameter of concentration revealed a significant effect of group; however, neither the effect of sample nor Group by Sample interaction were significant (Table 1). The AD group had significantly less concentrated stop clustering relative to the control group (Fig. 5C).

Movement trajectories varied in length under dark conditions in the open field and were sorted into long, medium, and short progression classes to analyze movement topography (Fig. 6A, B). The repeated measures ANOVA conducted on progression path circuity revealed a significant effect of class and Group by Class interaction; however, the effect of group was not significant (Table 2). Generally, as progression length increased paths become more circuitous; however, this relationship was more pronounced in the AD group (Fig. 6C).

Progression peak speeds varied in magnitude under dark conditions (Fig. 7A, B). The repeated measures ANOVA conducted on progression peak speed revealed a significant effect of class; however, the effect of group and Group by Class interaction was not significant

Table 1. Statistical data is shown for open field measures at two months. (*$p < 0.05$).

		F	df	p	η^2_p
Dark					
Distance					
	Group	13.168	(1, 31)	< 0.001*	0.298
	Sample	14.092	(1, 31)	< 0.001*	0.313
	Group × Sample	0.022	(1, 31)	0.882	7.161e-4
Stop time					
	Group	0.662	(1, 31)	0.422	0.021
	Sample	29.713	(1, 31)	< 0.001*	0.489
	Group × Sample	0.584	(1, 31)	0.451	0.018
Change in heading					
	Group	2.074	(1, 31)	0.160	0.063
	Sample	0.671	(1, 31)	0.419	0.021
	Group × Sample	8.199e-4	(1, 31)	0.977	2.645e-5
First order *r*	Group	4.373	(1, 31)	0.045*	0.124
	Sample	3.445	(1, 31)	0.073	0.100
	Group × Sample	0.517	(1, 31)	0.478	0.016
Light					
Distance					
	Group	1.401	(1, 31)	0.246	0.043
	Sample	3.506	(1, 31)	0.071	0.102
	Group × Sample	0.188	(1, 31)	0.668	0.006
Stop time					
	Group	0.089	(1, 31)	0.768	0.003
	Sample	3.485	(1, 31)	0.071	0.101
	Group × Sample	0.111	(1, 31)	0.742	0.004
Change in heading					
	Group	1.563	(1, 31)	0.221	0.048
	Sample	2.529	(1, 31)	0.122	0.075
	Group × Sample	0.078	(1, 31)	0.781	0.003
First order *r*	Group	0.106	(1, 31)	0.747	0.003
	Sample	3.223	(1, 31)	0.082	0.094
	Group × Sample	0.174	(1, 31)	0.680	0.006

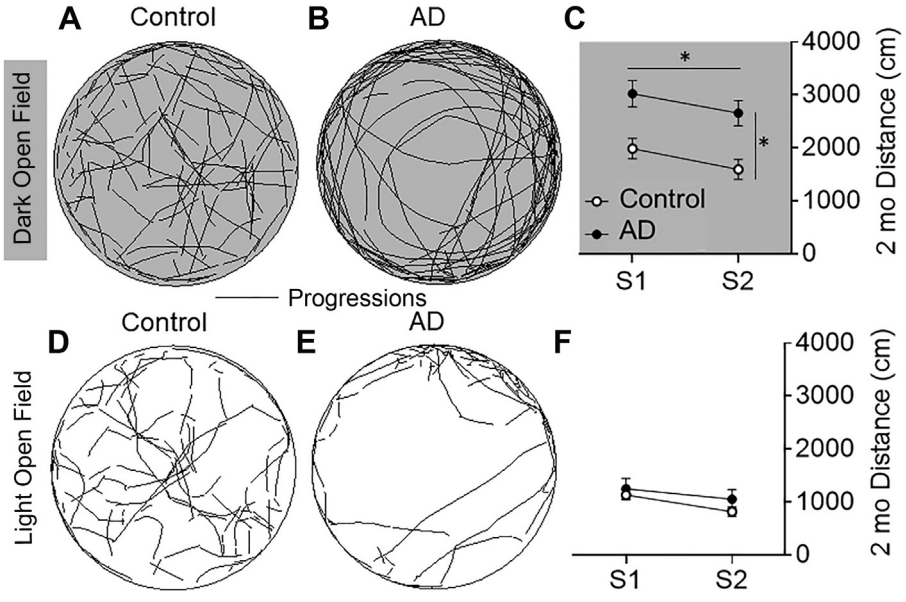

Fig. 2. Progressions traveled are displayed for the entire open field session at two months of age for a representative control and AD mouse. Line breaks represent where stops occurred along paths. At two months, AD mice traveled further distances under dark conditions than control mice **(C)**. Representative progressions are also shown for a control **(D)** and AD **(E)** under light conditions at two months of age. Progressions were organized similarly under light conditions **(F)**.

(Table 2). Progression peak speeds increased with progression class length (Fig. 7C).

Light conditions. During the light session at two months of age, mice fell off the table more frequently than in dark conditions at this age. Control mice fell off the table an average of 0.353 (min = 0; max = 2) times and AD mice fell off the table an average of 2.125 (min = 0; min = 5) times. An Independent samples t-test conducted on falls revealed that AD mice exhibited significantly more falls than control mice under light conditions [$t_{31} = 4.390$, $p < 0.001$, $d = 1.529$].

Mice organize their open field behavior under light conditions into progressions (Fig. 2D, E). The repeated measures ANOVA

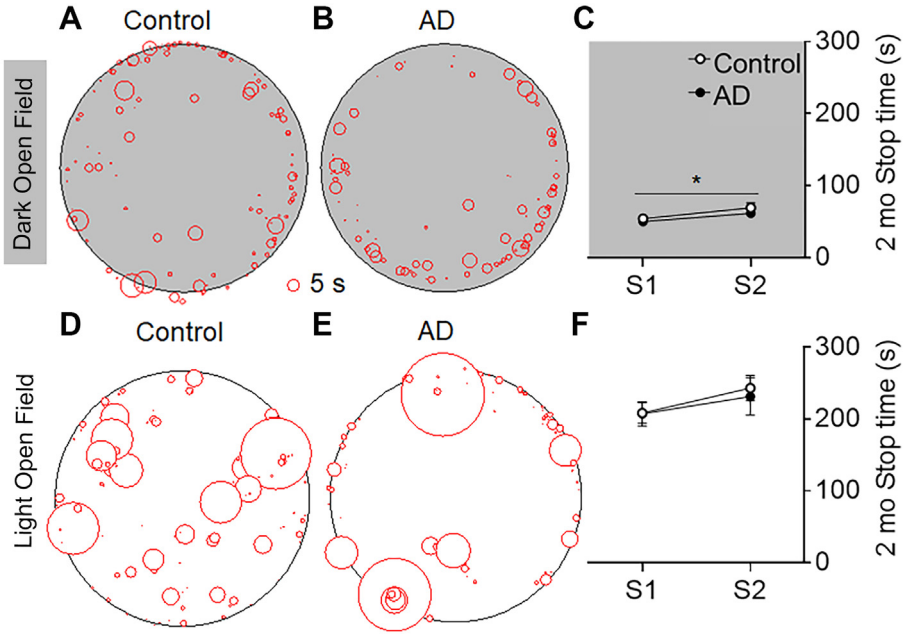

Fig. 3. Circular plots with stops are shown for a control **(A)** and AD **(B)** mice with stop durations represented by the size of the circle at two months of age. Stop time increased across the dark open field session at two months **(C)**, while no other differences were observed. Representative stops are displayed for one control **(D)** and one AD **(E)** mouse which failed to differ under light conditions at two months **(F)**.

conducted on total distance failed to reveal a significant effect of group, sample, or Group by Sample interaction (Table 1). No group differences were observed in the total distance travelled and distance travelled did not significantly vary across samples (Fig. 2F).

Mice exhibited stops of varied duration throughout the environment under light conditions (Fig. 3D, E). The repeated measures ANOVA conducted on total stop time failed to reveal a significant effect of group, sample, and Group by Sample interaction (Table 1). No group differences were observed in time spent stopping, and time spent stopping did not significantly vary across samples (Fig. 3F).

Stops occurred near the edge of the table under light conditions at two months. The percent of stops that occurred along the outer most 10% of the table was 63% for control and 60% for AD mice at two months under light conditions. No differences were observed between groups in the percentage of stops that occurred around the periphery of the table [$t_{31} = -0.322$, $p = 0.750$, $d = -0.112$].

Most of the change in heading during open field behavior under light conditions occurred during stops (Fig. 4D, E). The repeated measures ANOVA conducted on change in heading failed to reveal a significant effect of group, sample, and Group by Sample interaction (Table 1). Groups did not differ in change in heading (Fig. 4F), and no differences were observed across samples.

The location and duration or stop clustering within the open field varied light dark conditions. The parameter of concentration is a measure of stop clustering density within a direction (Fig. 5D, E). The repeated measures ANOVA conducted on parameter of concentration failed to reveal a significant effect of group, sample, or Group by Sample interaction (Table 1). Groups did not vary in stop clustering (Fig. 5F), and no differences were observed across samples.

Movement trajectories varied in the length under light conditions in the open field and were sorted into long, medium, and short progression classes to analyze movement topography (Fig. 6D, E). The repeated measures ANOVA conducted on progression path circuity revealed a significant effect of class; however, neither the effect of group nor the Group by Class interaction was significant (Table 2). As progression length increased paths become more circuitous; however, this relationship did not differ between groups (Fig. 6F).

Progression peak speeds varied in magnitude under light conditions (Fig. 7D, E). The repeated measures ANOVA conducted on progression peak speed revealed a significant effect of class; however, the effect of group and Group by Class interaction were not significant (Table 2). Progression peak speeds increased with progression class length (Fig. 7F).

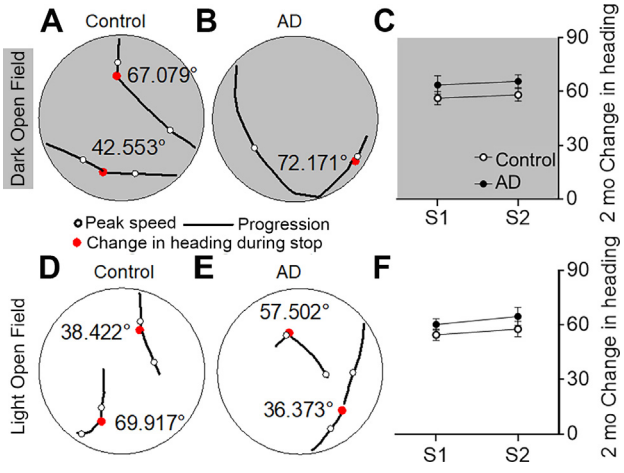

Fig. 4. Topographic plots with representative changes in heading for a control **(A)** and AD **(B)** mouse demonstrate similar organization of movement at two months of age under dark conditions **(C)**. Representative changes in heading are shown for a control **(D)** and AD **(E)** mouse under light conditions at two months that also shown similar movement organization **(F)**.

Open field behavior at four months

Dark conditions. During the dark session at four months, mice fell off the table infrequently. Control mice

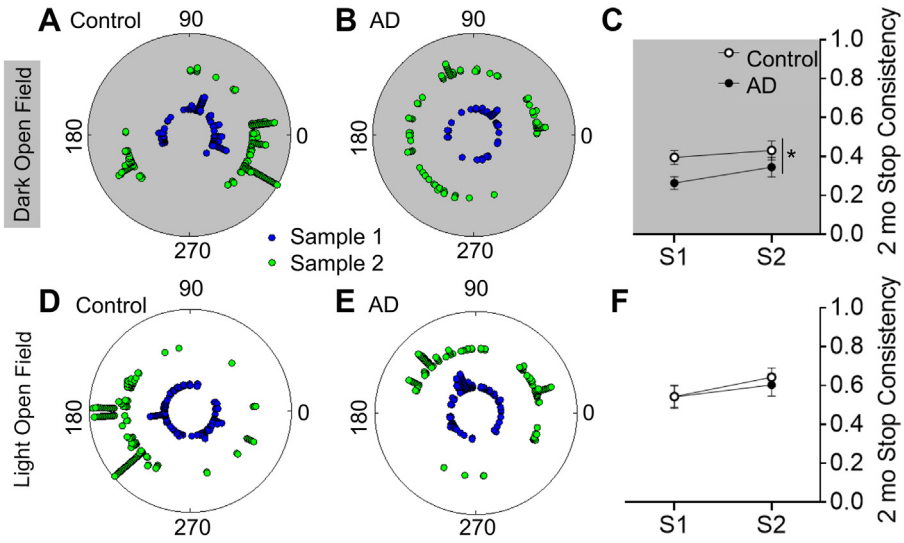

Fig. 5. Circular statistics displayed across two eight-minute samples for a representative control **(A)** and AD **(B)** mouse at two months old show that control mice engaged in more consistent stops under dark conditions relative to AD mice at two months **(C)**. However, control **(D)** and AD **(E)** mice exhibited similar stop consistency between groups under light conditions at four **(F)** months of age. (*$p < 0.050$).

fell off the table an average of 0.778 (min = 0; max = 4) times, and AD mice fell off the table an average of 1.125 (min = 0; min = 3) times. Groups did not significantly differ in falls at four months under dark conditions [$t_{15} = 0.667$, $p = 0.515$, $d = 0.324$].

Mice organized their open field behavior under dark conditions into progressions at four months of age (Fig. 8A, B). The repeated measures ANOVA conducted on total distance revealed a significant effect of group and sample; however, the Group by Sample interaction was not significant (Table 3). AD mice traveled significantly shorter distances relative to control mice (Fig. 8C). A significant decrease in distance travelled was observed across samples.

Mice exhibited stops of varied duration throughout the environment under dark conditions (Fig. 9A, B). The repeated measures ANOVA conducted on total stop time failed to reveal a significant effect of group, sample, or Group by Sample interaction (Table 3). Groups did not differ in total stop time, and no differences were observed between samples (Fig. 9C).

Stopping behavior occurred near the edge of the table under dark conditions at four months. The percent of stops that occurred along the outer most 10% of the table was 70% for control and 76% for AD mice at four months under dark conditions. No differences were observed between groups in the percentage of stops that occurred around the periphery of the table [$t_{15} = 0.209$, $p = 0.837$, $d = 0.102$].

Most of the change in heading during open field behavior under dark conditions occurred during stops (Fig. 10A, B). The repeated measures ANOVA conducted on change in heading revealed a significant effect of group; however, neither sample nor Group by Sample interaction were significant (Table 3). AD mice exhibited significantly larger change in heading relative to control mice (Fig. 10C), and no differences were observed between samples.

The location and duration or stop clustering within the open field varied under dark conditions (Fig. 11A, B). The repeated measures ANOVA conducted on parameter of concentration revealed a significant effect of group; however, neither the effect of sample nor Group by Sample interaction were significant (Table 3). The AD group had significantly more concentrated stop clustering relative to the control group (Fig. 11C), and no differences were observed between samples.

Movement trajectories varied in length under dark conditions in the open field and were sorted into long, medium, and short progression classes to analyze movement topography (Fig. 12A, B). The repeated measures ANOVA conducted on progression path circuity revealed a significant effect of group; however, neither the effect of class nor the Group by Class interaction was significant (Table 4). The AD group had significantly more circuitous progression relative to the control group (Fig. 12C), and no differences were observed between samples.

Progression peak speeds varied in magnitude under dark conditions (Fig. 13A, B). The repeated measures ANOVA conducted on progression peak speed revealed a significant effect of group and class; however, the

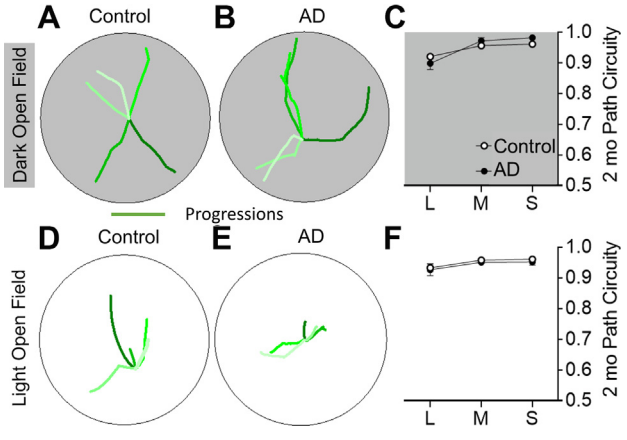

Fig. 6. Representative topographic plots with five progressions transformed to begin at a 0,0 origin are shown for a control **(A)** and AD **(B)** mouse that demonstrate similar organization of path circuity at two months of age under dark conditions **(C)**. Similar plots are displayed for control **(D)** and AD **(E)** mice under light conditions at two months. Under light conditions, no differences in path circuity were observed at two **(F)** months. (*$p < 0.050$).

Table 2. Statistical data is displayed for measures characterizing progression class at two months. (*$p < 0.05$).

		F	df	p	η^2_p
Dark					
Path circuity					
	Group	0.155	(1, 31)	0.697	0.005
	Class	26.458	(1, 31)	< 0.001*	0.460
	Group × Class	3.197	(1, 31)	0.048*	0.093
Peak speed					
	Group	3.108	(1, 31)	0.088	0.091
	Class	167.623	(1, 31)	< 0.001*	0.844
	Group × Class	2.549	(1, 31)	0.086	0.076
Light					
Path circuity					
	Group	0.408	(1, 31)	0.527	0.013
	Class	4.413	(1, 31)	0.016*	0.125
	Group × Class	0.007	(1, 31)	0.993	< 0.001
Peak speed	Group	2.419	(1, 31)	0.130	0.072
	Class	220.303	(1, 31)	< 0.001*	0.877
	Group × Class	2.884	(1, 31)	0.063	0.085

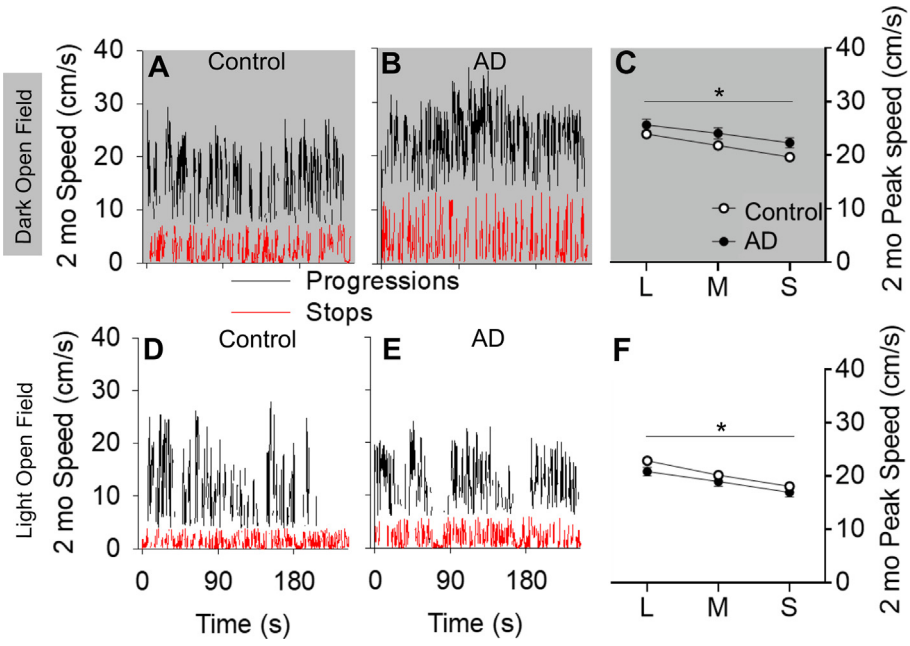

Fig. 7. Representative kinematic plots demonstrating moment-to-moment speeds (cm/s) for control **(A)** and AD **(B)** mice show similar organization of speeds traveled. Peak speed decreased across the dark open field at two months under dark conditions **(C)**. Representative speed plots are displayed for a control **(D)** and AD **(E)** mouse under light conditions at two months. All mice exhibited a decrease in peak speed throughout the light session **(F)**. (*$p < 0.050$).

Group by Class interaction was not significant (Table 4). Progression peak speeds were slower in the AD mice and increased with progression class length (Fig. 13C).

Light conditions. During the light session at four months of age, mice fell off the table less frequently than under dark conditions. Control mice fell off the table an average of 0.333 (min = 0; max = 1) times, and AD mice fell off the table an average of 0.500 (min = 0; min = 1) times. Groups did not significantly differ in falls at four months under light conditions [t_{15} = 0.664, p = 0.517, d = 0.323].

Mice organized their open field behavior under light conditions into progressions (Fig. 8D, E). The repeated measures ANOVA conducted on total distance revealed a significant effect of sample; however, neither the effect of group nor Group by Sample interaction were significant (Table 3). No group differences were observed in the total distance travelled, and distance travelled significantly decreased across samples (Fig. 8F).

Mice exhibited stops of varied duration throughout the environment under light conditions (Fig. 9D, E). The repeated measures ANOVA conducted on total stop time failed to reveal a significant effect of group, sample, and Group by Sample interaction (Table 3). No group differences were observed in stop duration, and time spent stopping did not significantly vary across samples (Fig. 9F).

Stops occurred around the table edge under light conditions at four months. The percent of stops that occurred along the outer 10% of the table was 63% for control and 66% for AD mice at four months under light conditions. No differences were observed between groups in the percentage of stops that occurred around the periphery of the table [t_{15} = 0.209, p = 0.837, d = 0.102].

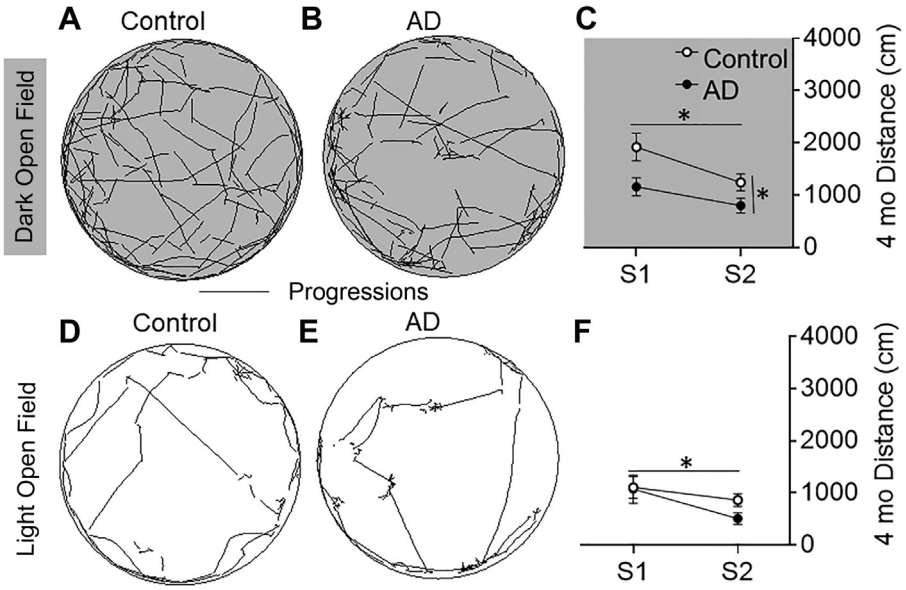

Fig. 8. Progressions are shown for one control **(A)** and AD **(B)** mouse at four months under dark conditions. Control mice traveled further distance than AD mice **(C)**. Representative progressions are displayed from a control **(D)** and AD **(E)** mouse under light conditions. All rats traveled shorter distance across the session, while on other differences were observed **(F)**. (*$p < 0.050$).

Most of the change in heading during open field behavior under light conditions occurred during stops (Fig. 10D, E). The repeated measures ANOVA conducted on change in heading failed to reveal a significant effect of group, sample, and Group by Sample interaction (Table 3). Groups did not differ in change in heading (Fig. 10F), and no differences were observed across samples.

The location and duration of stop clustering within the open field varied under light conditions (Fig. 11D, E). The parameter of concentration is a measure of stop clustering density within a direction. The repeated measures ANOVA conducted on parameter of concentration failed to reveal a significant effect of group, sample, or Group by Sample interaction (Table 3). Groups did not vary in stop clustering (Fig. 11F), and no

Table 3. Statistical data is presented for open field measures at four months. (*$p < 0.05$).

		F	df	p	η^2_p
Dark					
Distance					
	Group	6.779	(1, 15)	0.020*	0.311
	Sample	11.928	(1, 15)	0.004*	0.443
	Group × Sample	1.111	(1, 15)	0.308	0.069
Stop time					
	Group	0.015	(1, 15)	0.903	0.001
	Sample	2.842	(1, 15)	0.113	0.159
	Group × Sample	0.669	(1, 15)	0.426	0.043
Change in heading					
	Group	17.435	(1, 15)	< 0.001*	0.538
	Sample	0.902	(1, 15)	0.357	0.057
	Group × Sample	0.015	(1, 15)	0.903	0.001
First order *r*	Group	5.113	(1, 15)	0.039*	0.254
	Sample	0.178	(1, 15)	0.679	0.012
	Group × Sample	0.017	(1, 15)	0.898	0.001
Light					
Distance					
	Group	0.652	(1, 15)	0.432	0.042
	Sample	11.487	(1, 15)	0.004*	0.434
	Group × Sample	1.870	(1, 15)	0.192	0.111
Stop time					
	Group	0.003	(1, 15)	0.959	7.831e-4
	Sample	2.536	(1, 15)	0.132	0.145
	Group × Sample	0.055	(1, 15)	0.818	0.004
Change in heading					
	Group	0.953	(1, 15)	0.344	0.060
	Sample	1.259	(1, 15)	0.279	0.077
	Group × Sample	0.383	(1, 15)	0.545	0.025
First order *r*	Group	2.413	(1, 15)	0.141	0.139
	Sample	0.629	(1, 15)	0.440	0.040
	Group × Sample	0.287	(1, 15)	0.600	0.019

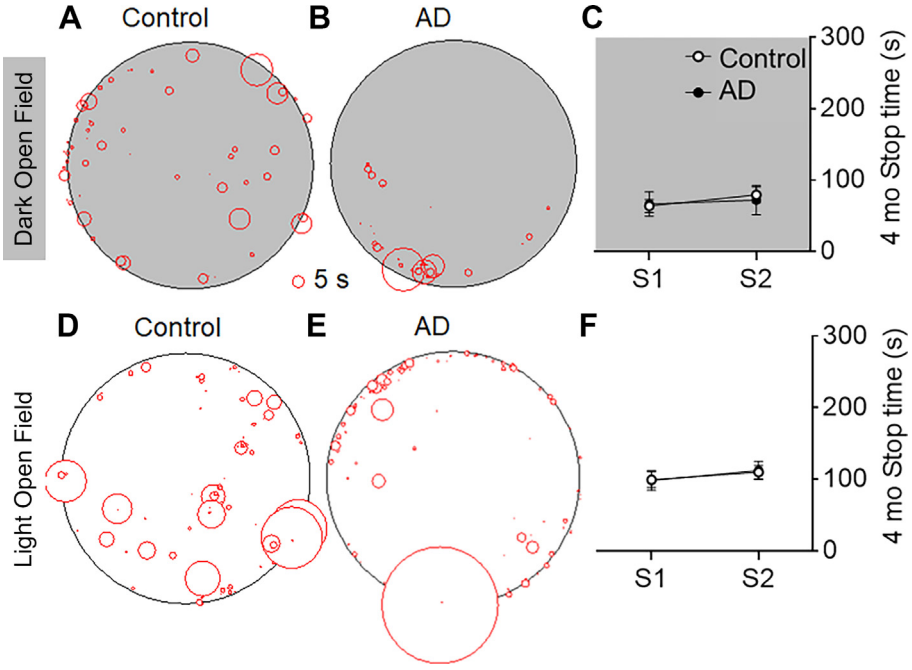

Fig. 9. Representative circular plots with stops (red circles) are shown for a control **(A)** and AD **(B)** mice at four months of age under dark conditions. Stop time increased across the dark open field session at four months **(C)**, while no other differences were observed. Representative stops are displayed for a control **(D)** and AD **(E)** mouse that failed to differ under light conditions at four months **(F)**. (*$p < 0.050$).

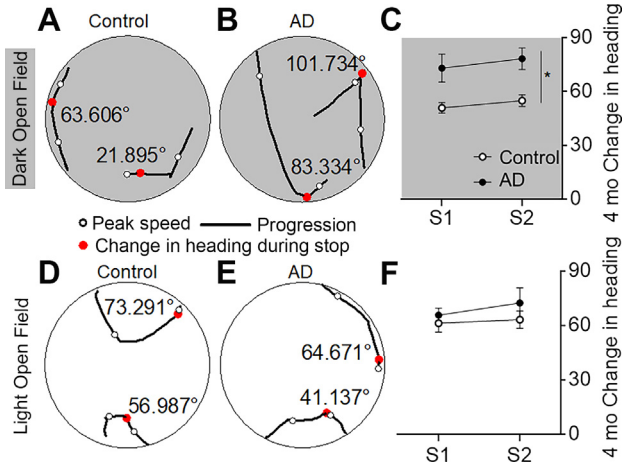

Fig. 10. At four months of age, topographic plots show that changes in heading were smaller for a representative control **(A)** mouse relative to a AD **(B)** mouse under dark conditions **(C)**. However, control **(D)** and AD **(E)** mice exhibited similar changes in heading under light conditions at four **(F)** months of age. (*$p < 0.050$).

differences were observed across samples.

Movement trajectories that varied in length under light conditions in the open field were sorted into long, medium, and short progression classes to analyze movement topography (Fig. 12D, E). The repeated measures ANOVA conducted on progression path circuity failed to reveal a significant effect of group, class, or the Group by Class interaction (Table 4). Groups did not vary in progression path circuity (Fig. 12F), and no differences were observed across samples.

Progression peak speeds varied in magnitude under light conditions (Fig. 13D, E). The repeated measures ANOVA conducted on progression peak speed revealed a significant effect of class; however, the effect of group and Group by Class interaction were not significant (Table 4). Progression peak speeds increased with progression class length (Fig. 13F).

DISCUSSION

The current study assessed hippocampal and cortical acetylcholinesterase and movement organization in the open field under dark and light conditions in the TgCRND8 mouse model of AD at two and four months of age. The AD modeled TgCRND8 mice exhibited spatial disruptions selectively during behavior in the dark open field at both ages, consistent with deficits in self-movement cue processing. Measures of performance differentially varied at both time points in these AD mice. These impairments in the dark open field were not associated with changes in optical densities of acetylcholinesterase. The results of this study demonstrate the ability of the open field task to test and dissociate different types of information processing that support spatial navigation in a genetic mouse model of AD. Disruptions observed in the organization of behavior in the dark open field are contextualized and the role of the hippocampal cholinergic system in spatial disorientation is evaluated while exploring other potential mechanisms for these deficits.

Locomotor activity

Aberrant locomotion has been reported in patients and mouse models with AD. Multiple reports indicate that hyperactivity is observed early in the progression of AD, followed by hypoactivity (for review see Zott et al., 2018). In TgCRND8 mice, results from locomotion studies have been mixed and are dependent on several factors, including sex, environmental enrichment, stress, anxiety, and age. For example, some work in TgCNRD8 mice has demonstrated normal activity levels relative to controls in one- to four-month-old female and male mice (Touma et al., 2004). In the present study, AD mice traveled further distances and faster peak speeds relative to control mice in the dark open field. Consistent with this work, under dark conditions, home cage activity has been shown to increase particularly during the dark cycle in TgCRND8 mice (Ambrée et al., 2006). Several other studies also support increased locomotor activity in this AD

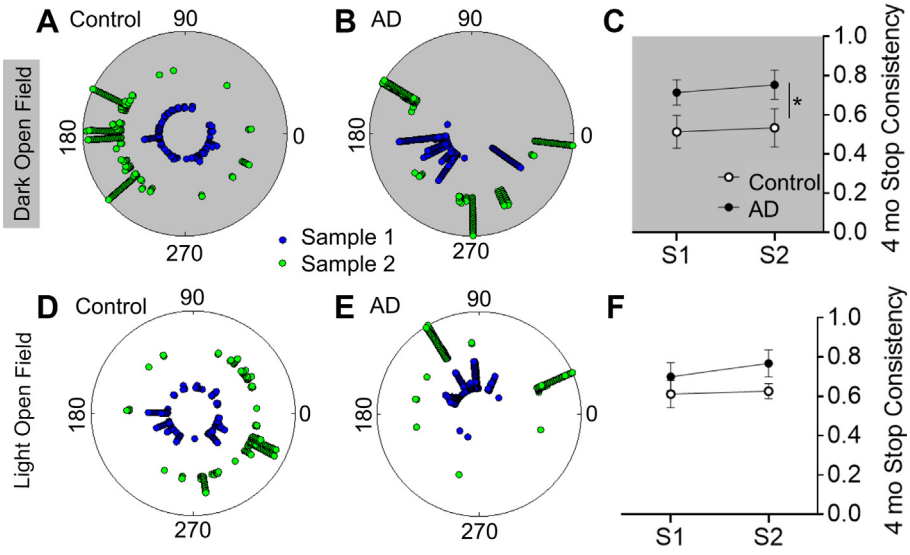

Fig. 11. At four months old, circular statistics plots for a control **(A)** and AD **(B)** mouse demonstrate that stops were more consistent for AD mice than control under dark conditions **(C)**. However, control **(D)** and AD **(E)** mice exhibited similar stop consistency between groups under light conditions at four **(F)** months of age. (*$p < 0.050$).

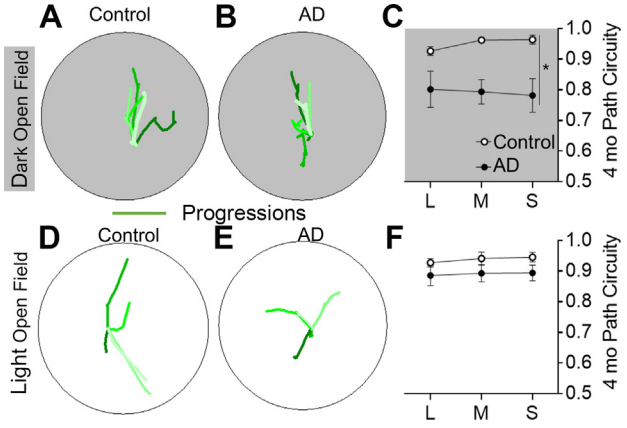

Fig. 12. Representative topographic plots with five progressions transformed to begin at a 0,0 origin are shown for a control **(A)** and AD **(B)** mice. At four-month-old under dark conditions, the control group traveled more direct paths relative to AD mice during long progressions **(C)**. (*$p < 0.050$).

mouse model (one- to four-month-old males; ~three- to five-month-old males (Richter et al., 2008); four- and nine-month-old females (Walker et al., 2011); two- to nine-month-old males (Hyde et al., 2005). Thus, hyperactivity may contribute to the changes observed in TgCRND8 mice during the dark open field session.

Stereotypic behavior can occur with AD. Patients with AD have been shown to engage in stereotypic ambulatory movements that can burden caregivers (Nyatsanza et al., 2003; Prioni et al., 2012). Stereotypy levels significantly correlate with corticosterone at 90 and 120 days old in TgCRND8 mice, suggesting increased anxiety (Ambrée et al., 2006). This work may suggest a role for anxiety influencing movement organization during the open field in TgCRND8 mice. Anxiety-like behavior in the dark open field would likely be related to stops along the periphery. However, the number of stops that occurred along the table periphery were similar between groups at both timepoints under dark and light conditions, suggesting anxiety may have not influenced movement organization during the dark open field in the current study. Therefore, increased locomotor activity observed in AD mice at two months of age in the current study is not likely attributed to increased anxiety relative to control mice but hyperactivity.

Spatial disorientation

Several disruptions in behavior solely in the dark open field were observed by TgCRND8 mice in the current study. First, more circuitous paths were exhibited at both ages by AD mice as well as differential impairments in stopping behavior. Second, further deficits were observed at four months of age with a subset of AD mice engaging in larger changes in heading. These results are consistent with deficits in self-movement cue processing (vestibular, motor efferent copies, proprioception, optic flow).

Under dark conditions, animals rely on self-movement cues to guide the organization of movement throughout the environment. In addition, depending on the availability of cues in the environment, different sources of information processing that guide navigation are hierarchically organized (Eichenbaum, 2017). Self-movement cues dominate under dark conditions and underlie navigation when access to environmental cues is available. Importantly, various forms of neuropathology can differentially impact the ability to use these sources of information to guide navigation.

Animals have an immense ability to compensate while navigating through space (Wallace et al., 2006; Allen et al., 2007; Martin and Wallace, 2007; Blankenship et al., 2017; Banovetz et al., 2021). Most of the work assessing spatial orientation in patients and rodent models of AD have focused on the use of visual environmental information to guide movement (Henderson et al., 1989; Tetewsky and Duffy, 1999; Duffy et al., 2004; Possin, 2010; Webster et al., 2014; Tuena et al., 2021). While these assessments are important, previous work with rodent models demonstrates that selective hippocampal pathology spares performance on these visuospatial tasks. Previous work assessing TgCRND8 mice in the water maze before four months of age failed to reveal spatial deficits (Touma et al., 2004; Görtz et al., 2008). Prior to the current study, spatial orientation had not yet been evaluated under dark conditions in a genetic mouse model of AD; this work demonstrates the importance of evaluating different sources of information processing in AD at early time points to detect select behavioral deficits and to track disease progression. Some spatial disrup-

Table 4. Statistical data is displayed for measures characterizing progression class at two months. (*$p < 0.05$).

		F	df	p	η^2_p
Dark					
Path circuity					
	Group	11.230	(1, 15)	0.004*	0.428
	Class	0.398	(2, 30)	0.675	0.026
	Group × Class	1.787	(2, 30)	0.185	0.106
Peak speed					
	Group	9.292	(1, 15)	0.008*	0.383
	Class	38.538	(2, 30)	< 0.001*	0.720
	Group × Class	1.729	(2, 30)	0.195	0.130
Light					
Path circuity					
	Group	2.415	(1, 15)	0.141	0.139
	Class	0.846	(2, 30)	0.439	0.053
	Group × Class	0.103	(2, 30)	0.902	0.007
Peak speed	Group	2.024	(1, 15)	0.175	0.119
	Class	108.174	(2, 30)	< 0.001*	0.878
	Group × Class	0.619	(2, 30)	0.545	0.040

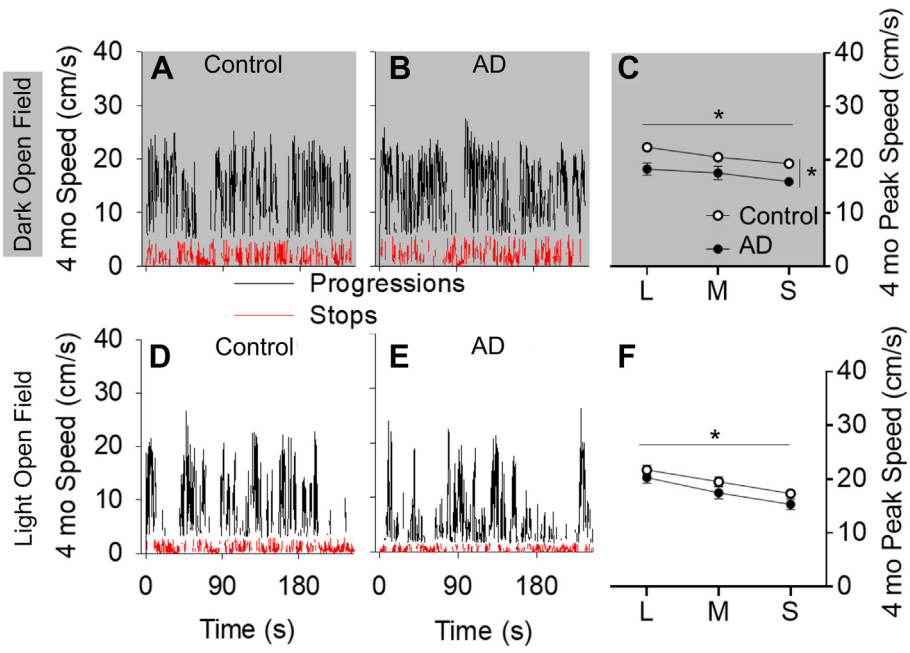

Fig. 13. At four months, control **(A)** mice traveled faster speeds compared to AD **(B)** mice under dark conditions **(C)**. Representative moment-to-moment speeds are shown for a control **(D)** and AD **(E)** mouse at four months under light conditions. Decreases in speed were observed across the light open field session for all rats at this timepoint **(F)**. (*$p < 0.050$).

tions have been identified during mild cognitive impairment preceding AD (Hort et al., 2007; Allison et al., 2016). Such early markers of AD will be essential for effective drug development, as early treatment will likely be essential to slow progression of the disease. By selectively manipulating access to different sources of information processing that animals use to navigate, spatial disorientation may be unveiled and dissociated before pathology spreads and becomes so severe it renders treatments ineffective.

Spatial disorientation often results from vestibular dysfunction. The vestibular system provides critical sources of information from horizontal and vertical head rotations that contribute to spatial navigation and balance (Fitzpatrick et al., 2006). TgCRND8 mice have been observed to engage in significantly more foot slips in a balance beam test and impaired gait at two months of age (Russo et al., 2018), suggesting an impaired vestibular system (Bent et al., 2004; Anson et al., 2019). Patients with mild cognitive impairment and AD commonly exhibit vestibular impairments that may contribute to spatial disruptions (Agrawal et al., 2019). For example, after 50 steps in an eyes-closed stepping task patients with AD were more likely to move and turn than stay in the same position (Nakamagoe et al., 2015). In addition, mice with varying severities of vestibular pathology exhibit disorientation during movement organization in the dark open field. Genetic mouse models (tilted mice (Blankenship et al., 2017) and Usher mice (Donaldson et al., 2018)) and mice with bilateral arsinilate acid lesions (Banovetz et al., 2021) engage in more circuitous paths, larger changes in heading, and less consistent stops in the dark open field, similar to TgCRND8 mice in the current study. Circling behavior is also commonly observed in both rodent models with vestibular pathology (Bloom and Hultcrantz, 1994; Ishiguro et al., 2007; Donaldson et al., 2018; Banovetz et al., 2021) and TgCRND8 mice (Janus et al., 2000; Ambrée et al., 2006; Granger et al., 2016). Similar circling behavior was observed in TgCRND8 mice in the current study within the home cage

and during testing sessions. Taken together, this may indicate that vestibular function is compromised in TgCRND8 mice at two months before the emergence of plaques and changes in acetylcholinesterase, contributing to spatial disorientation.

Neuropathology in TgCRND8 mice

Hippocampal pathology is sufficient to produce spatial disorientation depending on several factors. Broad hippocampal damage via electrolytic lesions of the fimbria fornix that impairs most inputs to the hippocampus disrupts rats' performance in the hidden platform version of the water maze, a standard task used to assess rodent spatial function (Whishaw et al., 1995). Selective 192-IgG Saporin lesions of the medial septum that cause hippocampal cholinergic deafferentation spares performance on this task but disrupts spatial orientation under dark conditions during food hoarding behavior (Martin and Wallace, 2007). Following these selective lesions, rats engaged in more circuitous paths and larger changes in heading. Spatial disorientation was also observed in a food hoarding task under dark conditions in rats following selective GABAergic GAT-1 lesions of the medial septum (Köppen et al., 2013). Taken together, this work demonstrates that select disruptions to multiple neural systems that may be compromised early in the progression of AD can influence movement organization under dark conditions.

Previous work demonstrated hippocampal changes throughout development in TgCRND8 mice that have the potential to contribute to spatial deficits. For example, brain volume increased at one-week of age in multiple structures, including the hippocampus, and remained significantly larger until 20 weeks of age (Allemang-Grand et al., 2015). Further, at these ages, TgCRND8 mice also exhibited the greatest density of Aβ plaques and reactive astrocytes, which abnormally increase in number due to nearby damaged neurons, in the enlarged brain regions. Additional work has shown a decrease in hippocampal neurons in TgCRND8 mice, with no changes in the total number of neurons and glial cells at three months (Steele et al., 2014). The current study leaves the contribution of specific neurotransmitters and neuropathology in question. It is likely that acetylcholinesterase was assessed too early or that it was not an appropriate cholinergic marker in the current study, as previous work has shown a reduction in circulating levels of acetylcholine in the cortex of male TgCRND8 mice at 28 weeks of age (Bellucci et al., 2006). The results of the current study suggest that TgCRND8 mice likely experience an aberrant neural response that changes throughout development as the disease progresses that may be sufficient to differentially influence general performance measures and disrupt behavior during the dark open field across development.

Multiple neural systems are disrupted in TgCRND8 mice. Though no changes were observed in optical densities of hippocampal acetylcholinesterase in the current study, other types of hippocampal pathology may have occurred to influence performance. For example, synaptic dysfunction has been reported in young TgCRND8 mice with decreased strength in the hippocampus (Hinrich et al., 2016) followed by hyperexcitability at nine and 20 weeks of age. Inhibitory GABA attenuation combined with increased glutamatergic function likely contributes to this hyperexcitability (Jolas et al., 2002; Mahar et al., 2017), followed by an overall reduction in GABA function at 24 weeks old (Jolas et al., 2002; Krantic et al., 2012; Ma and McLaurin, 2014; Albuquerque et al., 2015). Increasing work also supports a role for the noradrenergic system influencing spatial disorientation in AD, which is susceptible to degeneration with age and increased neuropathology in TgCRND8 mice (Francis et al., 2012). However, the degeneration of these neurotransmitter systems typically occurs later in the disease progression, after the spatial deficits observed in the current study were identified.

Neuroinflammation is still debated as a consequential or driving factor in AD. Plaques have been identified as early as nine to ten weeks of age in TgCRND8 mice, and the emergence of these plaques was accompanied by increased neuroinflammation (Dudal et al., 2004). An increased neuroinflammatory response early in life is likely related to aberrant neurogenesis that is static but not functional. These changes may be sufficient to impair hippocampal function, disrupt information processing related to self-movement cues, and interfere with behavior in the dark open field. It is possible that behavioral assessments have not been sensitive enough to detect performance changes which correlate with increases in APP, neuroinflammation, or other types of neuropathology throughout development that precede neurodegeneration. Further work is needed to fully understand the effects of neuropathology in TgCRND8 mice on spatial function early in development.

In conclusion, this study highlights the importance of dissociating the use of different sources of information to guide navigation when assessing spatial function in AD, as it is likely that wandering behavior is beginning earlier than currently detected. The current work characterized dark and light open field behavior in TgCRND8 mice at two and four months of age along with hippocampal and cortical acetylcholinesterase. At 28 weeks of age, TgCRND8 mice exhibit changes in cholinergic function (Hyde et al., 2005), though acetylcholinesterase optical densities did not change at two or four months of age, suggesting that other neural processes may be contributing to changes observed in the organization of behavior in the dark open field. A sequential analysis of progressions and stops in the open field provided a sensitive behavioral assessment of impairments under dark conditions. Two months of age is the earliest timepoint reported for spatial disorientation in TgCRND8 mice and provides the first documentation of disruptions in self-movement cue processing in a mouse model of AD. Furthermore, this is the first study to demonstrate a dissociation in performance dependent on the availability of cues in the environment, such that compensation in movement organization occurred under light conditions. Depending on the severity of neuropathology and the age of assessment, similar spatial disruptions

would be expected to be observed in movement organization in the dark and light open field in other mouse models of AD. This work provides an opportunity for future studies to investigate other mechanisms that contribute to spatial disorientation and potential treatments that may rescue function in AD at these early timepoints.

CONFLICT OF INTEREST/DISCLOSURE STATEMENT

The authors have no conflict of interest (financial or non) to report.

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