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Adult-born neurons in the hippocampus are essential for social memory maintenance

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Authors and affiliations: Elise C Cope[1], Renée C Waters[1], Emma J Diethorn[1], Kristen A Pagliai[1], Carla G Dias[1], Mumeko Tsuda[2], Heather A Cameron[2], Elizabeth Gould[1]*

[1]Princeton Neuroscience Institute and Department of Psychology, Princeton University, Princeton, NJ 08544 and [2]National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892

Author contributions: E.C.C., M.T., H.A.C. and E.G. designed research; E.C.C, R.C.W, M.T., H.A.C. and E.G wrote the paper; E.C.C., R.C.W., E. J. D., K.A.P., and C.G.D. performed research. E.C.C. and R.C.W analyzed data.

Correspondence should be addressed to Elizabeth Gould, Princeton Neuroscience Institute and Department of Psychology, Princeton University, Princeton, NJ 08540. E-mail: goulde@princeton.edu.

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Abstract

Throughout adulthood, the dentate gyrus continues to produce new granule cells, which integrate into the hippocampal circuitry. New neurons have been linked to several known functions of the hippocampus, including learning and memory, anxiety and stress regulation, and social behavior. We explored whether transgenic reduction of adult-born neurons in mice would impair social memory and the formation of social dominance hierarchies. We utilized a conditional transgenic mouse strain (TK mice) that selectively reduces adult neurogenesis by treatment with the antiviral drug valganciclovir (VGCV). TK mice treated with VGCV were unable to recognize conspecifics as familiar 24 hr after initial exposure. We then explored whether reducing new neurons completely impaired their ability to acquire or retrieve a social memory and found that TK mice treated with VGCV were able to perform at control levels when the time between exposure (acquisition) and re-exposure (retrieval) was brief. We then explored whether adult-born neurons are involved in dominance hierarchy formation by analyzing their home cage behavior as well as their performance in the tube test, a social hierarchy test, and did not find any consistent alterations in behavior between control and TK mice treated with VGCV. These data suggest that adult neurogenesis is essential for social memory maintenance, but not for acquisition nor retrieval over a short time frame, with no effect on social dominance hierarchy. Future work is needed to explore whether the influence of new neurons on social memory is mediated through connections with the CA2, an area involved in social recognition.

Significance Statement

Adult hippocampal neurogenesis has been implicated in behaviors linked to the hippocampus, including social behavior. We utilized a conditional transgenic mouse line to reduce adult-born neurons and explored social memory and social dominance hierarchy formation. We found that mice with reduced numbers of new neurons were unable to recognize conspecifics as familiar with a long delay after initial exposure but were able to recognize them as familiar with a short delay. We did not observe changes in social dominance as measured by home cage behavior or tube test performance in mice with reduced numbers of new neurons. These data confirm and extend previous reports to show that adult-born neurons are essential for maintenance but not for acquisition or short-term retrieval of social memories, nor for social dominance.

Introduction

New granule cells are continuously added to the rodent hippocampus throughout life (Jessberger and Gage, 2014; Kuhn et al., 2018). These new neurons form connections with inhibitory interneurons in the hilus, dentate gyrus, and the CA3 (Drew et al., 2016; Restivo et al., 2015) as well as with excitatory pyramidal cells in the CA3 and CA2 (Llorens-Martin et al., 2015; Toni et al., 2008). Accumulating evidence suggests that the CA2 region is involved in social recognition memory (Hitti and Siegelbaum, 2014; Stevenson and Caldwell, 2014). Studies have shown that lesions and optogenetic silencing of CA2 neurons impair, while optogenetic activation of these neurons improves, social recognition, indicating that these neurons are essential for encoding social information into memories (Hitti and Siegelbaum, 2014; Stevenson and Caldwell, 2014; Smith et al., 2016). Additional studies have shown that the CA2 region plays a role in social aggression (Pagani et al., 2015; Leroy et al., 2018). Since new neurons form connections with CA2 neurons (Llorens-Martin et al., 2015), their elimination may impair social behaviors linked to this region.

Adult neurogenesis has been implicated in many of the known cognitive functions of the hippocampus, including spatial memory, contextual fear memory, and pattern discrimination behavior (reviewed by Cope and Gould, 2019). There are also reports suggesting that adult-born neurons are important for social behavior, including social recognition memory (Garett et al., 2015; Monteiro et al., 2014; Pereira-Caizeta et al., 2017; Pereira-Caizeta et al., 2018) and social dominance (Opendak et al., 2016; Kozorovitskiy et al., 2004; Smagin et al., 2015). Using antimitotic drugs and gamma irradiation, a report has found that mice with reduced adult neurogenesis have impairments in 24 hr social recognition memory (Pereira-Caizeta et al., 2018). Studies using environmental enrichment to enhance adult neurogenesis have found social recognition memory persistence for up to 7 days and this effect is dependent on adult neurogenesis (Monteiro et al., 2014; Pereira-Caizeta et al., 2017). However, these studies found

behavioral effects earlier than the time frame required for new neurons to be functionally incorporated into the hippocampal circuitry and thus be relevant for behavior. Using a conditional doublecortin transgenic line to reduce immature neuron numbers by activation of diphtheria toxin, Garret et al (2015) showed that reduced adult neurogenesis impaired social recognition memory when a 2 hr interval between novel and familiar mouse exposure was used. We sought to replicate and extend these findings using a different transgenic model of adult neurogenesis reduction and examining whether mice lacking new neurons are completely incapable of social recognition memory even when the interval between exposure to the same conspecific is very short.

Reports have shown that social experiences can have both positive and negative effects on adult neurogenesis, depending on the context. For instance, negative social experiences such as social defeat stress, social isolation, and subordinate social rank position are associated with reduced levels of adult hippocampal neurogenesis (Lu et al., 2003; Lagace et al., 2010; Van Bokhoven et al., 2011; Opendak et al., 2016; Kozorovitskiy et al., 2004). Conversely, rewarding social experiences such as sexual experience, dominant social rank position and positive fighting experience are associated with higher levels of adult neurogenesis (Glasper and Gould, 2013; Opendak et al., 2016; Kozorovitskiy et al., 2004; Smagin et al., 2015). Yet, no work has explored whether reduced adult neurogenesis alters agonistic behaviors or dominance hierarchy formation.

We tested the hypothesis that reducing adult neurogenesis impairs social memory and social hierarchy formation. We utilized mice that express herpes simplex virus thymidine kinase (TK) under the control of the GFAP promoter. With treatment of the antiviral drug valganciclovir (VGCV), adult neurogenesis can be inhibited in TK mice. We found that mice with reduced numbers of adult-born neurons had no change in sociability but lacked social recognition when tested 24 hr after exposure to a previously encountered conspecific. We then reduced the delay

between the first and second exposure from 24 hr to 30 min and found that both TK and control mice treated with VGCV exhibited social recognition behavior that was similar to TK and control mice without VGCV. We next explored whether adult neurogenesis is important for social dominance hierarchy by examining home cage behavior and the non-aggressive tube test paradigm. We did not observe any significant changes in social dominance or agonistic behavior after VGCV treatment. Our data suggest that adult-born neurons are required for maintaining, and potentially retrieving, social recognition memories over longer time periods, but not for the acquisition or retrieval of such memories over shorter time frames.

Materials and Methods

Animals, experimental design, and VGCV treatment

All animal procedures were performed in accordance with the Princeton University animal care committee's regulations and were in accordance with the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals. Transgenic mice expressing herpes-simplex virus-thymidine kinase (TK) under the GFAP promoter were provided from the [Author Lab]. Heterozygous GFAP-TK mice were generated at [Author University] by crossing CD1 male mice with heterozygous GFAP-TK female mice. At PND15, mice were genotyped using Transnetyx. Both male and female CD1 and GFAP-TK mice were used for this study (n = 24 for each genotype with roughly equal numbers of mice of each sex). All mice were housed 4 per cage in Optimice cages on a reverse 12:12 light:dark cycle. For social memory testing, all mice were housed 4 per cage by sex. For home cage and tube test behavior, all mice were housed 4 per cage by genotype and sex. Littermates were housed together when possible. Individual animals within a cage were identified by unique brown hair dye markings. Beginning at ~6-7 weeks of age, home cage behavior and social memory were assessed as described

below (Figure 1). After this, mice of both genotypes were fed VGCV mixed in powdered chow (227 mg VGCV/kg chow) 5 days a week, alternating with standard pellet chow for 2 days. Social memory, home cage behavior, and social hierarchy in the tube test were then re-tested/tested after 6 weeks of VGCV treatment. This time-frame was chosen because it takes ~4-6 weeks after their production for new neurons to become functionally incorporated into the hippocampal circuitry and contribute to behavior (Denny et at., 2012; Kee et al., 2007). VGCV treatment continued throughout the rest of behavioral testing until perfusion.

Social memory testing

To assess social memory, the direct social interaction test was conducted in an open-field box (23 x 25 x 25 cm) in low light (10-20 lux) and during the active cycle for mice (dark). Each mouse underwent social memory testing 3 times (once prior to VGCV treatment and twice after 6 weeks of VGCV treatment). This test consists of 3 trials separated by either 24 hr or 30 min. Mice were first habituated to the behavior testing room for at least 30 min prior to testing. Mice were then habituated to the box for 5 min on the initial day of testing before each bout of behavioral testing. In trial 1, the test mouse and a never-before encountered mouse (novel mouse 1) were placed together in the open field box and allowed to interact for 5 min. Sex-matched young adult CD1 mice were used as novel mice. After this interaction period, the test mouse was returned to their home cage for either 24 hr or 30 min and then placed back into the open field arena with the mouse previously encountered in trial 1 (trial 2, familiar mouse A) and then a new, novel mouse from a different cage than the first novel mouse (trial 3, novel mouse 2). The time the test mouse spent interacting with the encountered mouse was measured from video recordings for each trial. Social interaction was defined as anogenital or nose-to-nose sniffing, following, or allogrooming that was initiated by the test mouse. In some instances, fighting was observed between the test mouse and novel conspecific. If this occurred, the trial was ended and the animal was not included in the behavior analyses. The excluded mice were:

1 CD1 male mouse in the 24 hr delay after VGCV treatment study and 3 male mice from each genotype from the 30 min delay after VGCV treatment study.

Home cage behavior testing

Home cage behavior was recorded under red light for 3 hr starting at the beginning of the dark phase. Mouse behavior was observed from video recordings of the session. Videos were scanned for occurrences of agonistic behaviors, behaviors that involved physical conflict between animals. These behaviors include but are not limited to chasing, biting, mounting, fighting, freezing, escape behavior, and defensive behavior. All videos were watched in their entirety at 2-30x speed to identify if this behavior was present. For cages where agonistic behavior was observed, behavior was analyzed for 20 min after the first occurrence of an agonistic act of any mouse. The numbers of instances that an animal exhibited offensive behavior and elicited defensive behavior were recorded.

Social hierarchy tube testing

The tube test was used to measure social hierarchy as previously described (Fan et al., 2019). The tube test consisted of 3 phases: habituation to a tube, training to pass through a tube, and social hierarchy testing. Mice were habituated to a short (7.6 cm in length) clear tube (2.3 cm in diameter) in their home cage for 3 days prior to training. Training and testing were completed in low light and during the active cycle for mice (dark). Mice were habituated to the behavior room 30 min prior to testing. During training, mice were placed at the entrance of a clear tube (30 cm in length and 2.3 cm in diameter) and then trained to pass through it. Each mouse passed through the tube 10 times, 5 times through each side. To prevent mice from retreating from the tube, a plastic stick was used to guide the mouse forward if necessary. Following 2 days of training, social hierarchy testing began. On each day of testing, mice were first trained to walk through an empty tube 2 times, once from each side. Mice were then tested against each cage

mate in pairs using a randomized round-robin design. Mice were paired such that each mouse encountered every other mouse within that cage only once per day. Two cage mates were placed at opposite ends of the tubes simultaneously and were released once they met in the middle. The trial was completed when one of the opponents retreated out of the tube. Each cage mate pairing had a loser and a winner, with the loser counted as the first animal with all 4 paws retreated out of the tube and the winner counted as the animal passing forward through the tube. If neither mouse retreated within a 2 min period, mice were retested against each other after a brief rest in their home cage. Testing continued for 8 consecutive days and social rank for each animal was determined based on the number of wins for each day. The tube was cleaned after every mouse with 5% bleach in water followed by 70% ethanol.

Histology

Mice were anesthetized with Euthasol and then transcardially perfused with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) for immunolabeling with PSA-NCAM to detect immature neurons in the dentate gyrus. In the dentate gyrus, PSA-NCAM appears to label the same population of cells as doublecortin, a known marker of immature neurons (Nacher et al., 2001; Spampanato et al., 2012). Extracted brains were post-fixed for 48 hr in 4% PFA and then cryoprotected in 30% sucrose for an additional 48 hr. Unilateral coronal sections (40 µm) were collected throughout the entire rostrocaudal extent of the hippocampus using a cryostat (Leica CM3050S). Sections were pre-blocked in PBS containing 0.3% Triton X-100 and 3% normal donkey serum for 1.5 hr at room temperature. Sections were then incubated in pre-block solution containing antibodies rat anti-PSA-NCAM (1:400, BD Pharminogen) for 24 hr at 4°C. Washed sections were then incubated for 1.5 hr at room temperature in secondary antibodies consisting of goat anti-rat Alexa Fluor 568 (1:250; Invitrogen). Washed sections were counterstained with Hoechst 33342 (1:5,000, Molecular Probes), mounted onto slides, and coverslipped with Vectashield (Thermo Fisher). Slides were coded until completion of the data

analysis. Cell densities for immature neurons (PSA-NCAM) were analyzed on 3 neuroanatomically-matched sections of the dorsal dentate gyrus (Franklin and Paxinos, 2008) using a BX-60 Olympus microscope. The number of PSA-NCAM+ cells was determined using a 100X oil objective. The granule cell layer area was outlined at low power (4X objective) using Stereo Investigator software (MBF Bioscience). Cell densities were then determined for each animal by taking the total number of PSA-NCAM+ cells and dividing it by the volume of the granule cell layer (area multiplied by 40 for thickness of cut section). Representative sections were collected using a 20X objective on Leica TCS SP8 confocal.

Statistical analyses

All behavioral and histological analyses were performed by an experimenter who was blind to the experimental group. Data from the social memory experiments were first analyzed by three-way ANOVA (sex x genotype x trial). No significant interactions were observed in these analyses (Table 1), so the male and female data were combined and social investigation times were analyzed using a repeated measures two-way ANOVA (genotype x trial) followed by Bonferroni post hoc comparisons. Difference scores were analyzed using unpaired or paired Student's t-tests where appropriate. Because home cage agonistic behaviors were not normally distributed (as determined by Shapiro-Wilk test) and had unequal variances (as determined by Levene's test), home cage agonistic behaviors were analyzed using an unpaired Mann-Whitney test or a paired Wilcoxon test where appropriate. For this measure as well, no statistical differences of genotype were observed for either sex (Table 1), so the male and female data were combined. Pearson's correlation coefficient test was used to analyze the association between home cage agonistic behaviors and performance in the tube test. Cell densities were analyzed by unpaired student's t-test. All data sets are expressed as the mean ± SEM and statistical significance was set at $p<0.05$ with 95% confidence. GraphPad Prism 8.2.0 (GraphPad Software) was used for statistical analyses and graph preparations.

Results

Mice expressing the GFAP-TK transgene have normal social memory behavior

To assess whether the GFAP-TK transgene alters social memory, we first measured social memory using a 3-trial paradigm in CD1 and TK mice prior to receiving VGCV (Figure 2A). Mice were exposed to a novel mouse in trial 1 (novel 1, day 1) and then re-exposed to the same mouse in trial 2 (day 2). In trial 3, the mice are exposed to a new, novel mouse (novel 2, day 3). Social memory is measured by the subject mouse's reduced sniffing time with the previously encountered mouse in trial 2 and the increased sniffing time with a new novel mouse (novel 2) in trial 3. Repeated measures two-way ANOVA showed that time spent sniffing the stimulus mouse changed over the testing period (2-way ANOVA; effect of trial: $F_{(1.932,\ 83.08)} = 49.5$, $p < 0.0001$; effect of genotype: $F_{(1,43)} = 0.07489$, $p = 0.7857$; trial X genotype: $F_{(2,86)} = 0.2034$, $p = 0.8164$) (Figure 2B). Post hoc comparisons revealed that CD1 and TK mice had normal social memory such that they decreased their interaction times with a familiar mouse (CD1, $p < 0.0001$; TK, $p < 0.0001$) and increased their interaction times with a novel mouse (novel 2), although this did not quite reach significance for CD1 mice (CD1, $p = 0.0634$; TK, $p = 0.0361$). There was no difference in time sniffing between genotypes on any trial (novel, $p > 0.9999$; familiar, $p > 0.9999$; novel, $p > 0.9999$). Furthermore, the sniffing time difference scores were similar between genotypes (novel 1 minus familiar, $t_{(43)} = 0.3590$, $p = 0.7213$, familiar minus novel 2, $t_{(43)} = 0.6909$, $p = 0.4933$) (Figure 2C).

Pharmacogenetic reduction of adult neurogenesis impairs 24 hr social memory

To investigate whether adult-born neurons are important for social memory, we tested social memory in CD1 and TK mice after 6 weeks of VGCV treatment using the previously described 3-trial behavioral paradigm (Figure 3A). Repeated measures two-way ANOVA of time sniffing

showed an effect of trial and an interaction between trial and genotype (effect of trial: $F_{(1.905, 85.72)}$ = 8.528, p = 0.0005, effect of genotype: $F_{(1,45)}$ = 0.03717, p = 0.8480; trial X genotype: $F_{(2,90)}$ = 7.185, p = 0.0013) (Figure 3B). Post hoc comparisons revealed that CD1 mice treated with VGCV have normal social memory (novel 1 vs. familiar, p = 0.0002; familiar vs. novel 2, p = 0.0093), whereas TK mice treated with VGCV had reduced social memory such that they did not decrease their investigation times with a previously encountered mouse (p = 0.6463) nor increase their interaction times with a novel mouse (novel 2) (p = 0.5948). Compared to CD1 mice, the sniffing time difference scores were significantly reduced in TK mice (novel 1 minus familiar, $t_{(45)}$ = 3.009, p = 0.00429, familiar minus novel 2, $t_{(45)}$ = 3.575, p = 0.00085) (Figure 3C).

Pharmacogenetic reduction of adult neurogenesis does not alter short-term social memory acquisition or retrieval

We explored the possible role of adult-born neurons in social memory (e.g. acquisition, maintenance, or retrieval) by testing CD1 and TK mice treated with VGCV using the 3-trial paradigm, but with a shorter delay between trials (30 min) (Figure 4A). Repeated measures two-way ANOVA of time sniffing showed an effect of trial and an effect of genotype (effect of trial: $F_{(1.645, 64.16)}$ = 20.28, p < 0.0001, effect of genotype: $F_{(1,39)}$ = 5.925, p = 0.0196; trial X genotype: $F_{(2, 78)}$ = 2.006, p = 0.1414) (Figure 4B). Post hoc comparisons revealed that CD1 and TK mice treated with VGCV had normal social memory with a shorter delay between trials such that they decreased their interaction times with a familiar mouse (CD1, p < 0.0001; TK, p < 0.0001) and increased their interaction times with a novel mouse (novel 2), although this did not reach significance for the TK mice (CD1, p = 0.0273; TK, p = 0.0610). It is worth noting however that CD1 mice had lower time sniffing on trial 1 (novel 1, p = 0.0293) and trial 2 (familiar, p = 0.0420) compared to TK mice. The difference scores were similar between genotypes in novel 1 minus familiar ($t_{(39)}$ = 1.4166, p = 0.1645) and familiar minus novel 2 ($t_{(39)}$ = 0.9011, p = 0.3730) (Figure 4C).

We then compared both groups' performance on the 30 min delay paradigm to their performance on the 24 hr delay paradigm. The sniffing time difference scores of CD1 mice with a 30 min compared to a 24 hr delay were similar between groups (novel 1 minus familiar, $t_{(38)}$ = 0.129, p = 0.898, familiar minus novel 2, $t_{(38)}$ = 0.761, p = 0.451) (Extended Data Figure 4-1A). Compared to the 30 min delay paradigm, TK mice treated with VGCV showed an effect in the 24 hr delay paradigm. The sniffing time difference scores were significantly altered between the 24 hr and 30 min delay paradigms in TK mice (novel 1 minus familiar, $t_{(40)}$ = 3.705, p = 0.00639, familiar minus novel 2, $t_{(40)}$ = 2.309, p = 0.0262) (Extended Data Figure 4-1B).

Pharmacogenetic reduction of adult neurogenesis does not alter agonistic behavior or social hierarchy

To assess whether reducing the number of adult-born neurons alters social hierarchy, we examined home cage behavior before and after treatment with VGCV. Before VGCV, there were no agonistic acts (e.g., mounting, chasing, biting, fighting) observed in any of the 5 CD1 cages and in 4 of the 5 TK cages. The number of agonistic acts did not differ between genotypes ($U_{(38)}$ = 190, p > 0.9999) (Figure 5A). Following 6 weeks of VGCV treatment, we then measured home cage behavior again and identified agonistic acts in 2 of the 5 CD1 cages and 2 of the 5 TK cages. The number of agonistic acts did not differ between CD1 or TK mice after VGCV treatment ($U_{(38)}$ = 175.5, p = 0.293) (Figure 5A), nor was there a significant difference when comparing before or after VGCV treatment in either genotype (CD1: $W_{(38)}$ = 15, p = 0.0625; TK: $W_{(38)}$ = 6, p = 0.250).

The slight increase in agonistic acts between the pre-VGCV and post-VGCV monitoring times in CD1 mice may have been due to the increased age of the mice. Because we only observed minimal agonistic acts during the home cage recordings, we were unable to assess social dominance within the home cage. We then explored social hierarchy within a home cage using

the non-aggressive tube test paradigm (Figure 5B). We determined an animal's rank position based on the number of wins of each mouse against its 3 cage mates over an 8-day period, with the highest rank position being 3 wins per day (example from a single CD1 cage, Figure 5C). 2 of 5 CD1 cages and 3 of 5 TK cages had 4 consecutive days where animals remained in the same rank position, suggesting that they exhibited stable hierarchies (Extended Data Figure 5-1). However, in all cages but 1 TK cage with continued tube test testing, we observed changes in animal's rank position. We examined the average wins of animals belonging to each rank group from the previous day across testing days and did not find any overt differences between CD1 and TK mice (Figure 5D). We also explored the relationship between agonistic behavior in the home cage and wins in the tube test. Animals with higher levels of agonistic behavior in the home cage did not consistently correspond to a greater number of wins in the tube test. No significant correlation was observed between tube test wins (cumulative wins over the 8-day testing period) and agonistic acts in the home cage after VGCV treatment (CD1 + VGCV: r = 0.1132, p = 0.6347; TK + VGCV: r =-0.2966, p = 0.2041).

VGCV reduces the number of adult-born neurons in the dentate gyrus of TK mice

After perfusion, we verified that VGCV was effective in reducing adult neurogenesis in the dentate gyrus of TK mice compared to CD1 mice. Examination of immature neuronal marker PSA-NCAM revealed that TK mice treated with VGCV showed a robust decrease in the density of immature

neurons in the dorsal dentate gyrus ($t_{(22)}$ = 11.01, p < 0.0001) (Figure 6A,B). There was no difference in the volume of the dentate gyrus between CD1 or TK mice treated with VGCV ($t_{(22)}$ = 2.454, p = 0.808). It should be noted that because the design used in this study was within-subjects, we were not able to quantify immature neurons prior to VGCV treatment in TK mice. Previous findings suggest that TK mice do not differ in the number of new neurons in the

dentate gyrus from their CD1 littermates prior to VGCV treatment (Snyder et al., 2011; Briones et al., unpubl observations).

Discussion

We investigated social memory and social dominance hierarchy in transgenic mice with reduced adult neurogenesis. Unlike mice with normal levels of adult neurogenesis, we found that TK mice treated with VGCV investigated familiar mice as if they were novel 24 hr after the initial exposure. By contrast, mice with reduced adult neurogenesis were able to recognize novel conspecifics as familiar when the delay between initial exposure and the second exposure was brief (30 min). This suggests that new neurons are not necessary for the acquisition and retrieval of short-term social memories, but are involved in maintaining, and potentially retrieving, social memories over longer periods of time. We did not observe any changes in mice with reduced adult neurogenesis compared to controls in either agonistic behaviors or social dominance in a home cage or by tube test assessment. Collectively, these findings suggest that adult-born neurons are essential for aspects of social memory that last beyond a 30 min interval.

Some studies suggest that social dominance status influences adult neurogenesis in the dentate gyrus. Studies using a “visible burrow system” in rats, a spatially and socially complex laboratory habitat used to encourage the formation of a dominance hierarchy, have found that subordinate rats have fewer new neurons than social dominants (Opendak et al., 2016; Kozorovitskiy and Gould, 2004), a finding that is consistent with research in mice showing that “positive fighting” experience increases the number of new neurons in the dentate gyrus (Smagin et al., 2015). In a stable hierarchy, the enhancement of adult neurogenesis in dominant rats appears to be driven by increased mating behavior (Opendak et al., 2016; Kozorovitskiy and Gould, 2004). By contrast, adult rats living in an unstable hierarchy exhibit reductions in

adult neurogenesis that appear to be driven by stress caused by increased aggression (Opendak et al., 2016). These findings raise the possibility that adult-born neurons are involved in social dominance. Here, in combination with home cage agonistic behavior assessment, we utilized the tube test, which is a non-aggressive test of social dominance (Fan et al., 2019), to explore the potential relationship between adult neurogenesis and social dominance. We were unable to obtain definitive evidence that new neurons are involved in social aggression or in social dominance hierarchy formation. Despite previous work showing that CD1 mice form stable hierarchies (So et al, 2015), neither CD1 controls nor TK mice exhibited consistent results in the tube test, nor in agonistic acts within the home cage, rendering it difficult to determine social rank status. One explanation for this lack of hierarchy formation could be that mice were housed at weaning with their littermates, which likely minimized fighting throughout the study. More challenging home cage environments (e.g., increased competition for resources) that promote higher levels of agonistic behavior might be necessary to investigate the relationship between home cage behavior and dominance rank. In our study, we examined agonistic behavior in both males and females. Previous studies have shown that male and female mice both form dominance hierarchies but use different strategies to form and maintain them (Williamson et al., 2019). Moreover, while males engage in more overt acts of aggression than females, the overall number of agonistic acts is similar between sexes (Choleris et al., 2018; van den Berg, 2015). This claim is consistent with our agonistic behavior findings overall, but future studies should investigate whether TK mice with inhibited adult neurogenesis display alterations in agonistic behavior in response to resident-intruder challenges, which can be designed to stimulate increased aggression in both males (Olivier et al., 1995; Yohn et al., 2019) and females (Haug et al., 1986; Newman et al., 2019).

Our findings on social recognition memory are consistent with previous reports using several methods to block adult neurogenesis, including irradiation (Pereira-Caizeta et al., 2018),

406 antimitotic drugs (treatment with either the DNA-alkylating agent temozolomide or central
407 infusion of the mitotic blocker cytosine arabinoside) (Pereira-Caizeta et al., 2018), as well as a
408 tamoxifen-inducible diphtheria toxin doublecortin transgenic mice (Garrett et al., 2015). Our data
409 replicate these findings in a different transgenic mouse of adult neurogenesis reduction, and
410 extend these previous studies by showing that adult neurogenesis is not necessary for the
411 acquisition of social memory, nor for its retrieval after a short time period (30 min), as TK mice
412 treated with VGCV were able to acquire and retrieve social memories using this temporal
413 parameter. However, we found that adult born neurons are important for maintaining social
414 recognition when the interval between exposure to the same mouse was 24 hrs, suggesting that
415 the impairment results from an inability to maintain, and potentially retrieve, social memories
416 over longer periods of time. There are some reports showing that adult-born neurons are
417 important for spatial memory acquisition and retrieval (Gu et al., 2012; Tronel et al., 2015), yet
418 less is known about adult-born neurons and memory maintenance/consolidation. While the
419 dentate gyrus itself has been shown to be important for social memory retrieval (Leung et al.,
420 2018), the link between this brain region and social memory maintenance has not been
421 explored.

422 Previous studies suggest that sex differences in social learning exist in rodents (Choleris and
423 Kavaliers, 1999). These differences are not large at baseline (Choleris et al., 2013; Karlsson et
424 al., 2015), however, and mostly involve emerging sex differences in social memory after
425 hormone or drug treatment (Matta et al., 2017; Choleris et al., 2013). As noted above for
426 aggressive behavior, male and female mice seem to use different social strategies, but both
427 sexes exhibit comparable evidence of social recognition memory (Choleris et al., 2018; van den
428 Berg et al., 2015; Karlsson et al., 2015). Consistent with this observation, we detected evidence
429 of social recognition memory in untreated male and female CD1 and TK mice, but no significant
430 interaction when sex x genotype x trial was examined for any of the social memory experiments,

431 demonstrating that males and females both displayed similar social recognition memory within a
432 given experiment. It remains possible, however, that significant sex differences in the effects of
433 genotype on social recognition memory would emerge if females were tested at different stages
434 of the estrous cycle, which was not done in the present study. A previous report suggests that
435 social recognition memory is most robust in females when learning occurs during proestrus
436 (Sanchez-Andrade and Kendrick, 2011). This report raises the possibility that activation of new
437 neurons may be augmented by high levels of estrogen, which is consistent with findings that
438 immature neurons express estrogen receptors (Herrick et al., 2006) and that estradiol treatment
439 enhances the activation of new neurons after exposure to a spatial memory task (McClure et al.,
440 2013). Future studies should determine whether estrogen-induced enhancements in social
441 recognition memory in females (Phan et al., 2012) require new neurons.

442 One possible interpretation of our data is that the behavioral deficit in mice lacking adult
443 neurogenesis reflects a broader impairment in novelty or familiarity detection. Indeed, some of
444 our results lend support to this possibility in that TK mice with inhibited adult neurogenesis show
445 reduced investigation times between the first novel mouse exposure to the second novel mouse
446 exposure. These findings raise the possibility that TK mice with inhibited adult neurogenesis
447 may habituate to social interactions in general and become less interested in sniffing mice,
448 regardless of their novelty. Considered in the context of the broader literature, however, it
449 seems unlikely that adult neurogenesis is important for novelty detection in a more general
450 sense. In a modified novel object recognition test with a 3 min delay between trials, Denny et al.
451 (2012) found in mice lacking adult neurogenesis increased novelty exploration when a novel
452 object replaced a familiar object over repeated exposures to the familiar object. While we found
453 an increase in social investigation times in TK mice treated with VGCV in the first novel and
454 familiar trial of the 30 min delay paradigm that may support this interpretation, there was no
455 difference in investigation times in the initial novel trial between genotypes in the 24 hr delay

456 paradigm. Furthermore, other studies have not found impairments in novel object recognition
457 tests with 24 hr delays in between recognition trials in mice with reduced new neurons
458 (Jaholkowski et al., 2009; Goodman et al., 2010), suggesting adult neurogenesis may not be
459 necessary for intact novelty or familiarity detection. Reduced adult neurogenesis has been
460 shown to affect social preference such that rodents without adult neurogenesis spend more time
461 with familiar conspecifics over novel conspecifics (Opendak et al., 2016; Pereira-Caixeta et al.,
462 2018). This suggests that they are able to recognize mice, but prefer familiar conspecifics.
463 However, our data do not support this possibility as there was no difference in social exploration
464 times in the familiar trials compared to the novel trials with the 24 hr delay between exposure,
465 suggesting no recognition of familiar conspecifics. Collectively, our results likely indicate a more
466 specific deficit in social recognition memory.

467 In addition to the hippocampus, GFAP+ radial precursors also reside in the subventricular zone
468 where they migrate along the rostral migratory stream and populate the olfactory bulb (Garcia et
469 al., 2004). Pharmacogenetic ablation of adult-born neurons using VGCV in TK mice reduces
470 olfactory bulb neurogenesis (Singer et al., 2009). Although olfactory discrimination is the main
471 function of the olfactory bulb (Abraham et al., 2010), mice lacking adult neurogenesis in this
472 region have no differences in simple odor discrimination tasks (Li et al., 2018; Imayoshi et al.,
473 2008; Sakamoto et al., 2011). Importantly, in our study, we did not see differences in social
474 investigation times between genotypes during the initial novel trial of the longer delay paradigms
475 and we found intact social memory with a shorter delay in between trials. These findings
476 strongly suggest that deficits in olfactory function are not responsible for the inability of mice
477 lacking adult neurogenesis to exhibit evidence of social discrimination in the 24 hr paradigm.
478 Additional studies have provided evidence for the postnatal and adult generation of new
479 neurons in other brain regions associated with social behavior, including the amygdala,
480 hypothalamus, and nucleus accumbens (Ahmed et al., 2008; Staffend et al., 2014; Kokoeva et

al., 2007). While postnatal neurogenesis in these areas has been less extensively studied, it remains possible that VCGV treatment of TK mice inhibited neurogenesis in these regions, which may have contributed to the deficit in social memory we observed.

Adult-born neurons form connections with neighboring mature granule cells (Luna et al., 2019), with local inhibitory interneurons in the hilus, dentate gyrus, and CA3 (Drew et al., 2016; Restivo et al., 2015), as well as with pyramidal cells in the CA3 and CA2 (Llorens-Martin et al., 2015; Toni et al., 2008). One report linked the ventral CA3 region to social memory (Chiang et al., 2018), and a growing body of literature suggests an important role for the CA2 region in social memory (Hitti and Siegelbaum, 2014; Stevenson and Caldwell, 2014). Furthermore, the CA2, through its connections with the hypothalamus, has been shown to be involved in social aggression (Leroy et al., 2018). It is likely that alterations in the number of new neurons lead to fewer connections to their target cells in CA2. This, in turn, may alter the proper function of the CA2.

This raises questions about the possible mechanisms by which new neurons exert their effects on CA2-dependent behavior. One possibility is that reducing adult neurogenesis could alter neural activity patterns in the CA2 region. Young granule neurons are known to have enhanced synaptic plasticity (Schmidt-Hieber et al., 2004; Marín-Burgin et al., 2012; Ge et al., 2007) and respond with excitation to the neurotransmitter GABA (Ge et al., 2006). New granule neurons also activate local inhibitory circuits that promote strong feed-forward inhibition onto mature granule cells (Drew et al., 2016) and when activated by specific afferents inhibit mature granule cells directly (Luna et al., 2019). Genetic enhancement of adult neurogenesis leads to an overall decrease in excitability while elimination of adult neurogenesis by irradiation leads to an overall increase in excitability in the dentate gyrus (Ikrar et al., 2013). Adult-born neurons have also been shown to directly impact hippocampal network activity (reviewed by Tuncdemir et al., 2019). Ablation of adult neurogenesis by irradiation or by chemotherapeutic drugs reduces

neuronal oscillations in the dentate gyrus, particularly in the theta frequency (Nokia et al., 2012; Park et al., 2015). In addition to reducing theta, adult neurogenesis inhibition has been shown to increase gamma frequency bursts and synchronization of neuronal firing to these bursts in the dentate gyrus (Lacefield et al., 2012). Since reduced adult neurogenesis alters dentate gyrus neural activity, this is likely to lead to disrupted neural activity in the CA2 target region. Previous work has shown that CA2 neurons participate in generating gamma oscillations and sharp-wave ripples in the hippocampus (Alexander et al., 2018). Since these neural activity patterns are associated with cognition and social memory (Buzsáki, 2015, Alexander et al., 2017), future work should explore whether reducing adult neurogenesis leads to disrupted neuronal oscillations in the CA2.

Figure legends

Figure 1. Experimental timeline. Six-week old CD1 and GFAP-TK mice were examined for home cage behavior and social memory with a 24 hr delay in between trials. After behavioral testing, CD1 and GFAP-TK mice were treated with valganciclovir (VGCV) in powdered chow for 6 weeks. Mice were then re-examined for home cage behavior and social memory with a 24 hr delay in between trials at ~13 weeks of age. Mice then underwent social hierarchy testing using the tube test at ~17 weeks of age and then re-tested for social memory with a 30 min delay in between trials at ~19 weeks of age. Mice were perfused at the end of the study.

Figure 2. Mice expressing the GFAP-TK (TK) transgene have normal social memory behavior. **A)** Schematic of 3-trial social memory test. **B)** Both CD1 and TK mice without VGCV treatment decrease their investigation times for a familiar mouse and increase their investigation times for a new novel mouse (novel 2). **C)**There was no change in difference scores (novel 1 minus familiar or familiar minus novel 2) between genotypes. n = 22 for CD1 and n = 23 for TK. Bars represent mean + SEM. *$p < 0.05$.

Figure 3. Pharmacogenetic reduction of adult neurogenesis impairs long-term social memory. A) 280 Schematic of 3-trial social memory test with a 24 hr delay between trials. B) Compared to CD1 mice treated with VGCV, GFAP-TK (TK) mice treated with VGCV do not decrease their investigation times for a familiar mouse and do not increase their investigation times for a new novel mouse (novel 2). C) TK mice treated with VGCV have lower difference scores compared to CD1 mice treated with VGCV. n = 23 for CD1 and n = 24 for TK. Bars represent mean + SEM. *p < 0.05.

Figure 4. Pharmacogenetic reduction of adult neurogenesis does not alter short-term social memory. A) Schematic of 3-trial social memory test with a 30 min delay between trials. B). Both CD1 and GFAP-TK (TK) mice with VGCV treatment decrease their investigation times for a familiar mouse and increase their investigation times for a new novel mouse (novel 2). C) There

was no change in difference scores (novel 1 minus familiar or familiar minus novel 2) between genotypes treated with VGCV. n = 20 for CD1 and n = 21 for TK. Bars represent mean + SEM. *p < 0.05. For comparison of mice with 24 hr delay and 30 min delay paradigms, see extended data figure 4-1.

Figure 5. Agonistic home cage behavior and tube-test ranking for social hierarchy in CD1 and GFAP-TK (TK) mice. A) Number of agonistic acts between CD1 and TK mice did not differ before or after VGCV treatment. B) Schematic of the tube test. C) Example of the wins of one cage tested daily over 8 days. Each data point indicates one animal in the cage (n = 1 cage with 4 mice). D) Summary graph (n = 5 cages/genotype) of the average wins of animals belonging to each rank group from the previous day in CD1 (left) and TK (right) mice. Error bars represent SEM. For individual cage tube test rankings, see extended data figure 5-1.

Figure 6. GFAP-TK (TK) mice treated with VGCV have fewer new neurons compared to CD1 mice treated with VGCV. **A)** PSA-NCAM immunolabeling in the dentate gyrus from CD1 and TK mice treated with VGCV (PSA-NCAM=red, Hoechst=blue). Scale bar = 75 μm. **B)** The density of PSA-NCAM+ neurons in the dentate gyrus was lower in TK mice treated with VGCV. n = 12 per genotype. Error bars represent SEM. *p < 0.05.

Extended Data Figures

Extended Data Figure 4-1. TK mice have impairments in social memory with a 24 hr delay, but not with a 30 min delay between testing phases. **A)** There was no change in difference scores (novel 1 minus familiar or familiar minus novel 2) between CD1 mice treated with VGCV when the delay is 24 hr or 30 min in between testing trials. n = 23 for CD1 + VGCV (24 hr) and n = 20 CD1 + VGCV (30 min). **B)** VGCV-treated TK mice have lower difference scores with 24 hr

delays compared to VGCV-treated TK mice with 30 min delays. n = 24 for TK+VGCV (24 hr) and n = 21 for TK + VGCV (30 min). Error bars represent SEM. *p<0.05.

Extended Data Figure 5-1. Tube test ranking for social hierarchy in individual cages of CD1 and TK mice. Wins of each CD1 **(A)** and TK **(B)** cage of mice tested daily over 8 days. Each data point indicates one animal in the cage with 4 mice per cage.

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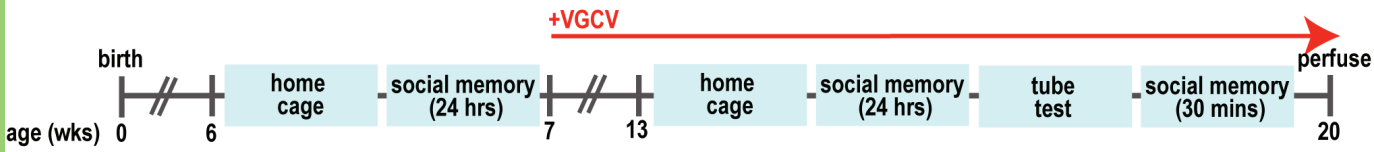

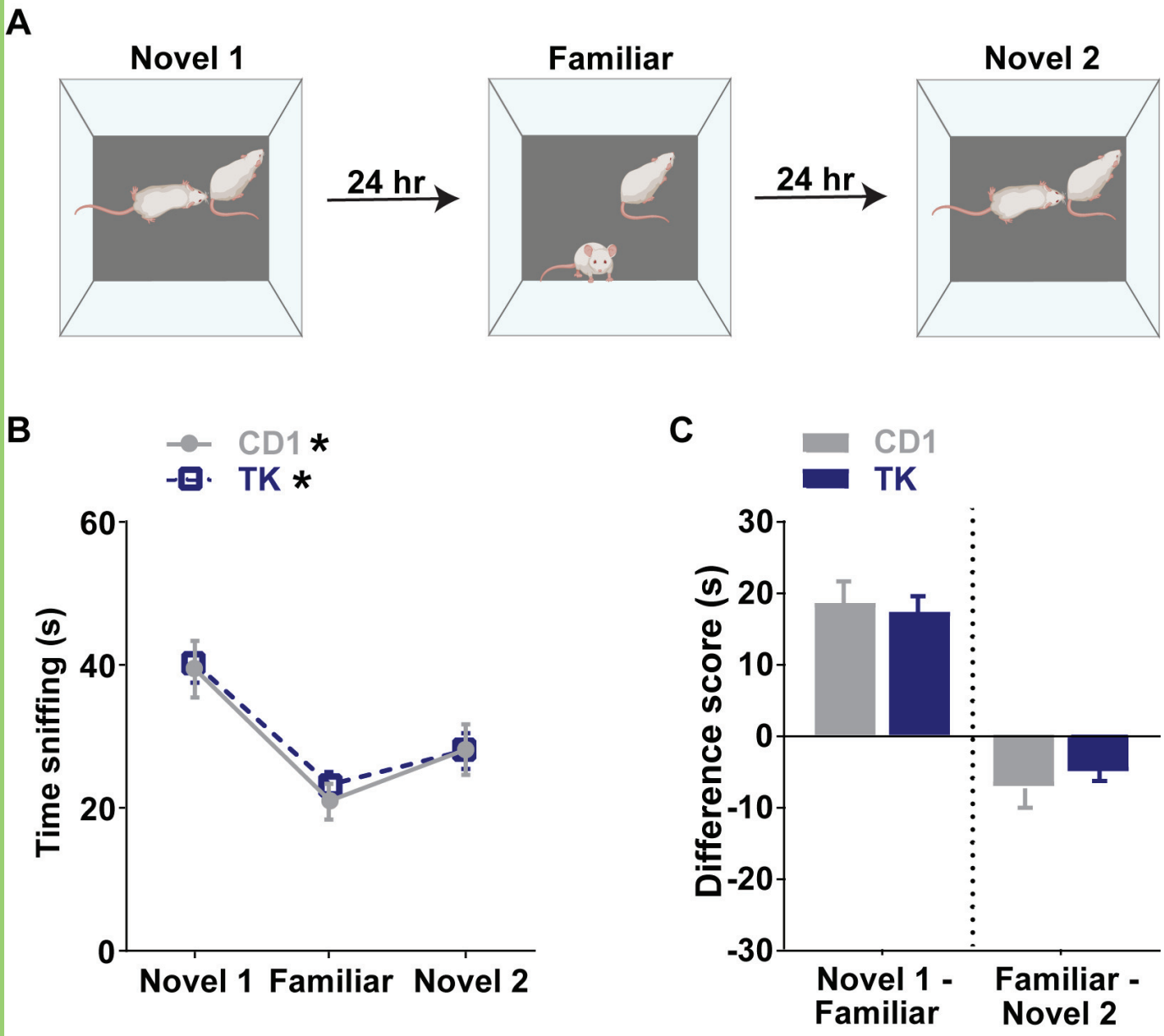

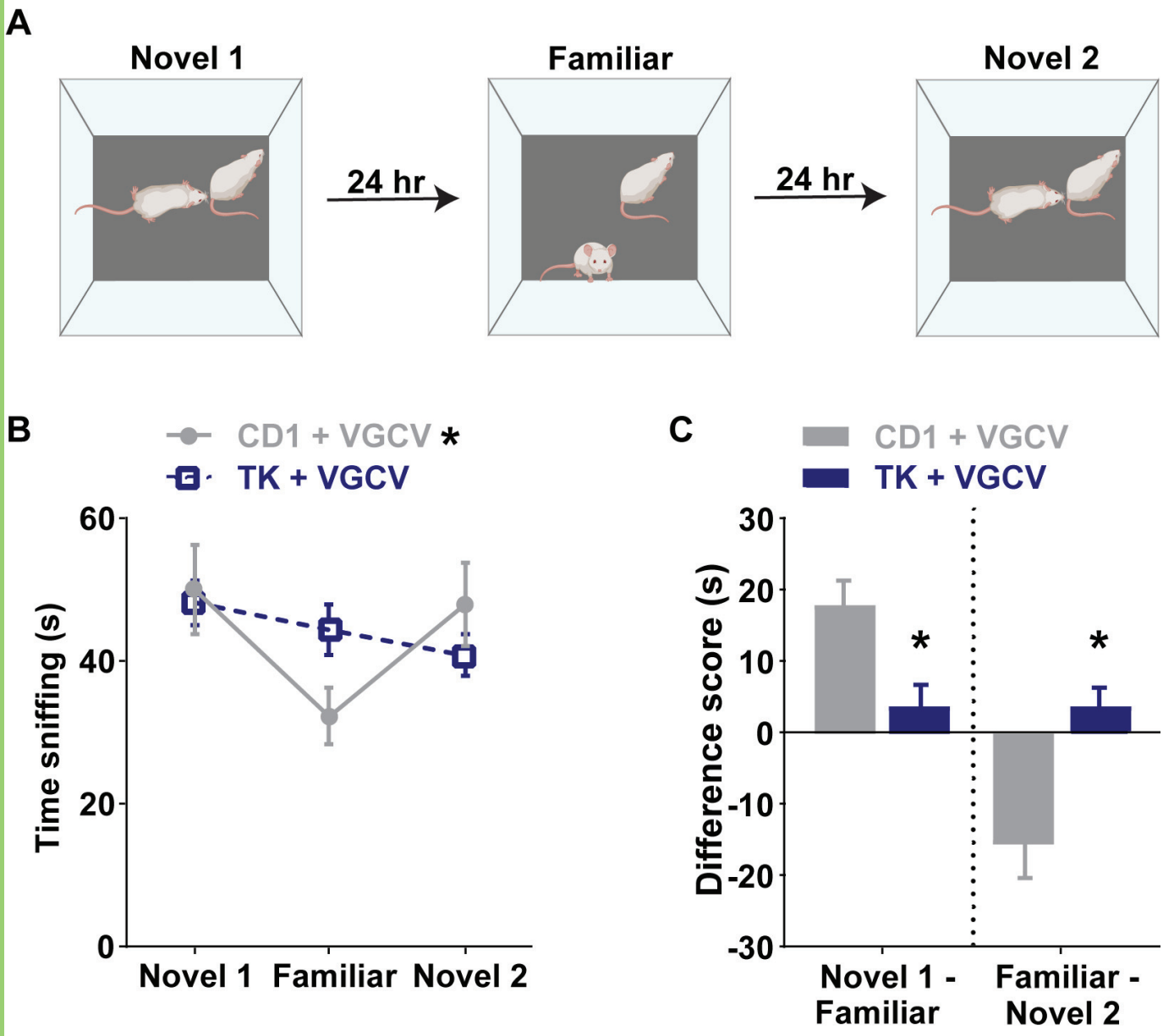

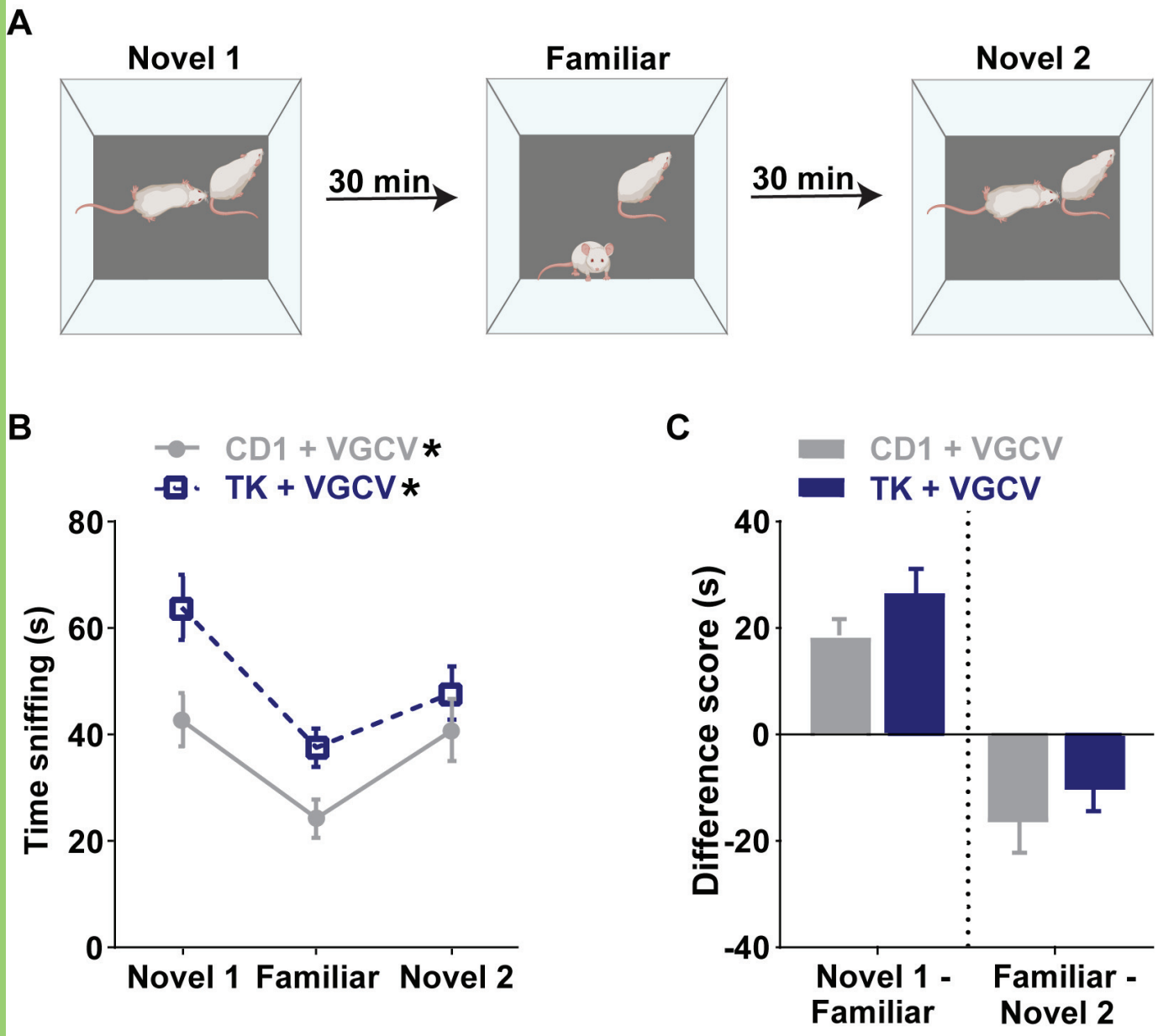

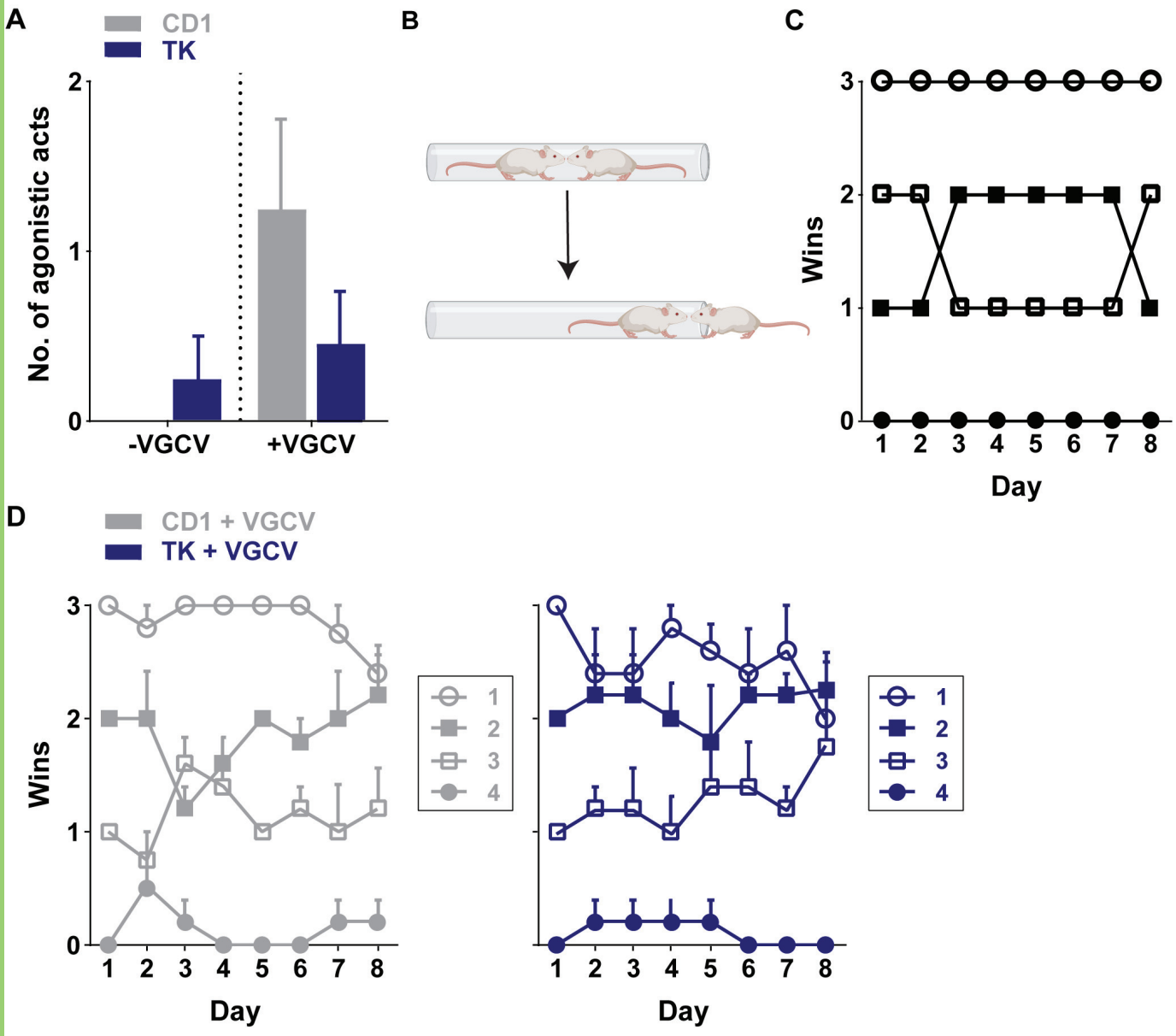

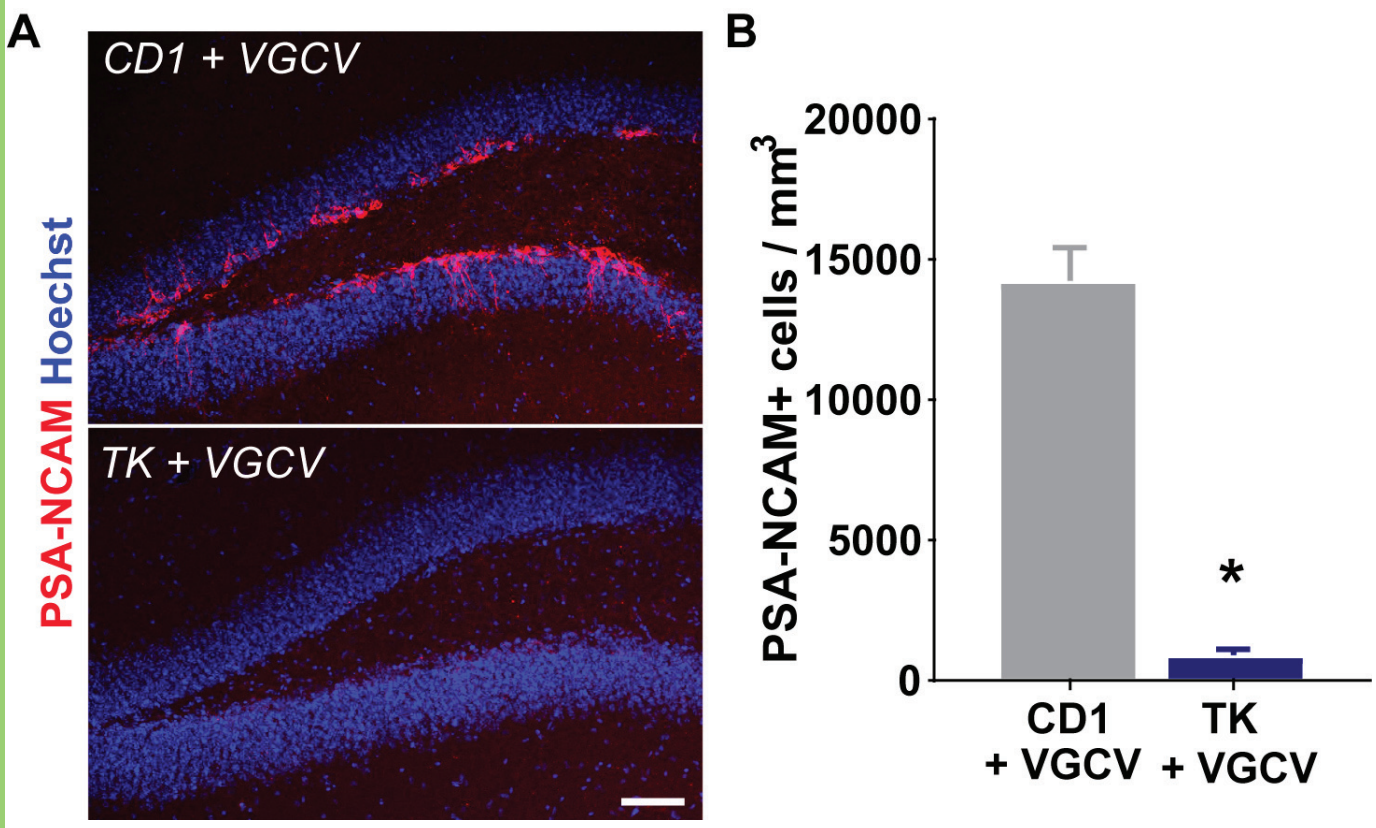

Table 1: Statistics for female/male comparisons in overall effect of genotype on behavior

Experiment	Statistics	Results
Social investigation pre-VGCV (24 hr)	three-way ANOVA	sex x genotype x trial: $F_{(2,82)} = 1.269$, $p = 0.2865$
Social investigation post-VGCV (24 hr)	three-way ANOVA	sex x genotype x trial: $F_{(2,86)} = 0.0807$, $p = 0.9226$
Social investigation post-VGCV (30 min)	three-way ANOVA	sex x genotype x trial: $F_{(2,74)} = 0.7855$, $p = 0.4596$
Agonistic behavior difference scores	Mann Whitney	♀ CD1 vs. TK: $U_{(18)} = 40$, $p = 0.4947$ ♂ CD1 vs. TK: $U_{(18)} = 31.50$, $p = 0.1089$

Statistical analyses demonstrating no overt differences between males and females in the effects of genotype on social behavior measures. Social memory data were analyzed using three-way ANOVA because the datasets met the criteria for parametric statistics. Agonistic behavior data were analyzed using Mann Whitney U tests, because this was not the case. In all experiments, the effects of genotype with or without VGCV were similar in females and males.