

Neuronal Excitability

Sprague-Dawley Rats Differ in Responses to Medial Perforant Path Paired Pulse and Tetanic Activation as a Function of Sex and Age

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Abstract

Network plasticity in the medial perforant path (MPP) of adult (five to nine months) and aged (18–20 months) urethane-anesthetized male and female Sprague Dawley rats was characterized. Paired pulses probed recurrent networks before and after a moderate tetanic protocol. Adult females exhibited greater EPSP-spike coupling suggesting greater intrinsic excitability than adult males. Aged rats did not differ in EPSP-spike coupling but aged females had larger spikes at high currents than males. Paired pulses suggested lower GABA-B inhibition in females. Absolute population spike (PS) measures were larger post-tetani in female rats than male rats. Relative population spike increases were greatest in adult males relative to females and to aged males. EPSP slope potentiation was detected with normalization in some post-tetanic intervals for all groups except aged males. Tetani shortened spike latency across groups. Tetani-associated NMDA-mediated burst depolarizations were larger for the first two trains in each tetanus in adult males than other groups. EPSP slopes over 30 min post-tetani predicted spike size in female rats but not in males. Replicating newer evidence MPP plasticity in adult males was mediated by increased intrinsic excitability. Female MPP plasticity was related to synaptic drive increases, not excitability increases. Aged male rats were deficient in MPP plasticity.

Key words: age-dependent; entorhinal cortex; granule cell; long-term potentiation; sex differences; short-term plasticity

Significance Statement

The medial perforant path (MPP)-dentate gyrus (DG) granule cell synapse was the site of discovery of long-term potentiation (LTP) in the mammalian nervous system but considering the current interest in sex and aging, surprisingly few studies have directly examined these variables in relation to tetanus-induced long-term and short-term (paired pulse) plasticity. Using an interleaved current-paired pulse interval protocol and moderate tetanic protocol young (five to nine months) and old (18–20 months) male and female urethane-anesthetized rats were found to differ in levels of granule cell intrinsic excitability, E-S coupling, GABA-B inhibition, and tetanic NMDA current contributions to post-tetanic population spike (PS) potentiation. This study provides a platform for future examination of sex-related and age-related changes in MPP-DG function.

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Introduction

In 1973, Bliss and Lomo published a landmark paper demonstrating tetanus-induced long-term potentiation (LTP) in medial perforant path (MPP)-evoked potentials recorded in the dentate gyrus of anesthetized rabbit (Bliss and Lomo, 1973). This paper became the starting point for hypotheses concerning the physiological basis of learning and memory. The enduring plasticity Bliss and Lomo found most frequently was reduced population spike (PS) latency, but increases in population EPSPs generated the greatest interest. Increases in PS amplitude were similarly frequent, but not consistently correlated with EPSP increases. Bliss and Lomo conclude that two independent mechanisms were responsible for MPP-LTP: (1) an increase in the efficacy of synaptic transmission and (2) an increase in the excitability of the granule cell population. The preponderance of experimental LTP investigations in dentate gyrus focused on the (1) mechanism, EPSP potentiation. However, in the last two decades, attention has turned to excitability increases. In 2016, Lopez-Rojas et al. (2016) presented evidence that an increase in dendritic intrinsic excitability is primarily responsible for dentate gyrus MPP-LTP in mature granule cells of male rats. A comparative review of EPSP and intrinsic excitability changes in learning and memory (Daoudal and Debanne, 2003) highlights the commonality of their induction mechanisms with both depending on NMDA receptors. Further, both types of plasticity events may act bi-directionally, increasing or decreasing connectivity, in neural networks. Sex and age differences have been understudied in MPP plasticity. The present set of experiments address those variables in the context of Bliss and Lomo's original observations.

Materials and Methods

Male and female Sprague Dawley rats (Charles River) were housed doubly in individually ventilated cages (Techniplast) with regular enrichment, on a reversed light cycle 12/12 h cycle (lights off at 7 A.M.) until the age of approximately two to four months, and then singly housed thereafter. Rats were fed regular chow (Teklad2018); however, both male and female rats were placed on a modest food restriction schedule at two to three months of age, to maintain a healthy aging weight profile and to reduce obesity related illnesses in old age (Hubert et al., 2000). Rats were fed between 8:30 and 10:30 A.M. daily and received an amount of food that was 75% (g) of the average age-

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We dedicate this article to the memory of Dr. Carolyn W. Harley who passed away November 24, 2022, prior to its completed publication. Her infectious enthusiasm, encouragement, and substantial scientific contributions shaped the lives and careers of a vast number of trainees and colleagues alike.

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dependent and sex-dependent *ad libitum* consumption (Hubert et al., 2000). Water was available *ad libitum*. The average mass at the time of electrophysiological recording in the five- to nine-month-old rats; females 366.33 ± 27.45 g and males 674.8 ± 38.89 g, and for 18- to 20-month-old rats; females 456.00 ± 81.82 g and males, 748.80 ± 71.11 g.

All experimental procedures occurred within the dark phase of the animals' light cycle (9 A.M. to 5 P.M.) and were performed in accordance with the Canadian Council of Animal Care (CCAC) guidelines and approved by the Memorial University of Newfoundland and Labrador Institutional Animal Care Committee.

Electrophysiological recording

At 5–9 or 18–20 months of age, rats were anesthetized with urethane (intraperitoneally). To reduce overdose susceptibility because of age, sex, or food restriction (Davis et al., 2000), rats were stepped up to a ~ 1.5 g/kg dose (15% w/v). Once anesthetized the rats were placed in a stereotaxic instrument in the skull flat position and body temperature was maintained at $37 \pm 0.5^\circ\text{C}$ via a feedback-regulated heating pad (FHC). A concentric bipolar stimulating electrode (NE-100; Kopf) was lowered into the PP ($\sim 7.2 \pm 0.3$ mm posterior, and $\sim 4.1 \pm 0.1$ mm lateral to bregma, and ~ 3.0 mm ventral from brain surface) and a glass pipette (0.9% NaCl, 1–3 M Ω) was lowered into the DG ($\sim 3.5 \pm 0.2$ mm posterior and $\sim 2.0 \pm 0.2$ mm lateral, and ~ 2.5 mm ventral; adjusted for animal size). A stainless-steel jeweler's screw (Fine Science Tools) served as ground. A 0.2-ms square unipolar test pulse was delivered (0.1 Hz) to the PP and the DG responses via a constant current stimulation unit (NeuroData Instruments) were amplified and filtered 1–10 kHz (Grass Instruments), digitized at 10 kHz, and stored online using SciWorks 9.0 or 11.0 software (Datawave Technologies). The stimulating and recording electrodes were then adjusted in the dorsal/ventral plane so that a maximal positive going waveform (granule cell layer) was achieved. See graphical experimental procedures in [Figure 1](#) for outline of experimental procedures and analyses.

Current intensity E-S coupling and paired pulse analyses

At the commencement of the experiment, an input-output (I-O) current intensity curve (100–1000 μA , 100- μA steps) was performed using a series of three paired pulses presented every 10 s at each current level. Sets of paired pulses consisted of one presentation each of interstimulus intervals (ISIs) 15, 70, and 120 ms such that each current level tested each ISI before the current was increased. Similar I-O and E-S profiles were reported in Walling and Harley (2004) in awake male rats using I-O current stimulation (0.1 Hz; 50–1000 μA) without paired pulse assays suggesting that paired pulse procedures did not alter I-O or E-S relationships. The paired pulse intervals were chosen to probe GABA-A sensitive paired pulse inhibition (PPI; 15 ms), paired pulse facilitation (PPF; 70 ms) and GABA-B late paired pulse inhibition (PPI; 120 ms) of the PP-DG evoked PS in urethane-anesthetized male (Joy and

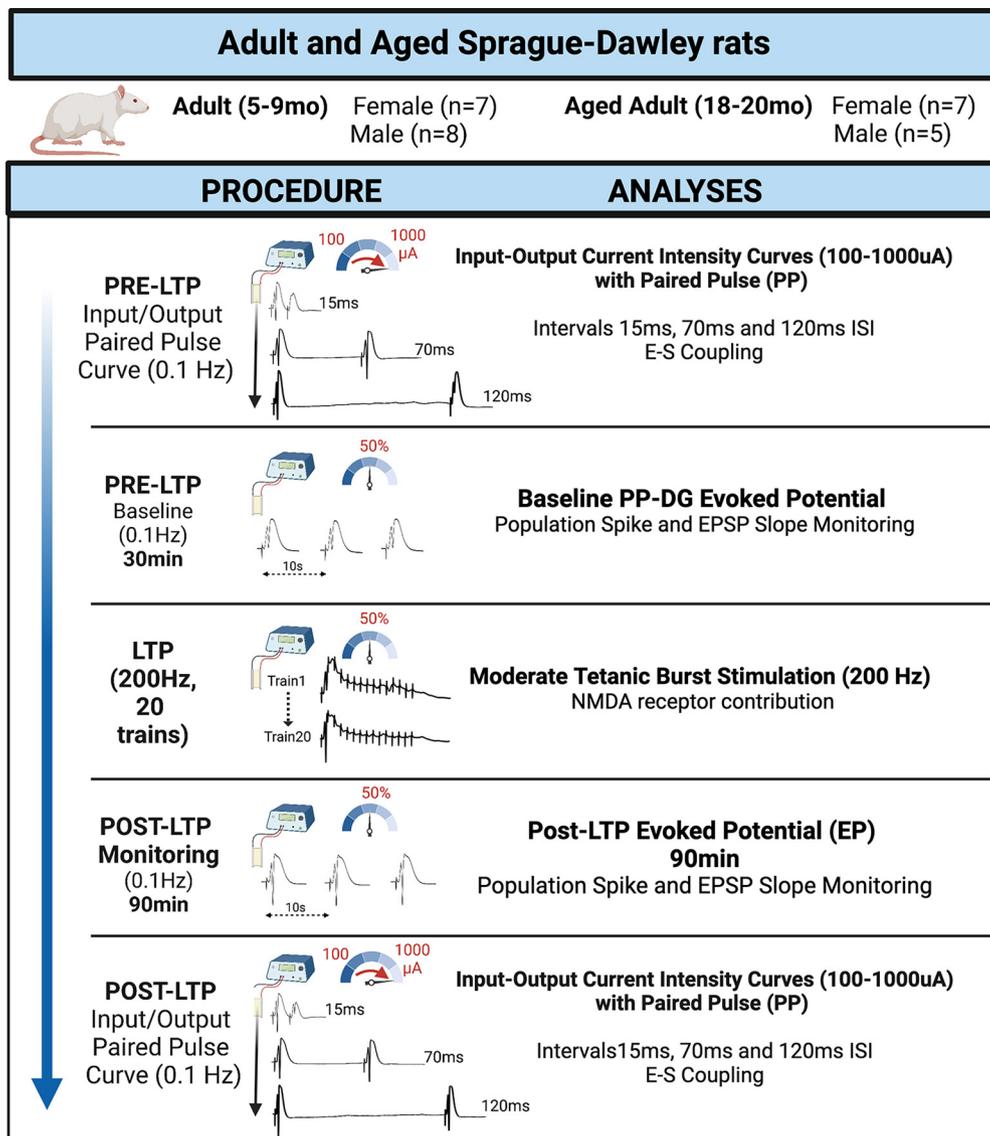


Figure 1. Graphical experimental procedure. In brief, experiment commenced with paired pulse (15, 70, and 120 ms interleaved on a current intensity profile; 100–1000 μ A). Baseline recording (min 30 min) with current eliciting 50% maximal PS. Moderate strength tetanic stimulation (Protocol B, [Straube and Frey, 2003](#)), followed by 90-min recording (0.1 Hz). Post-LTP PP and input-output current intensity curve. Image BioRender.

[Albertson, 1993](#); [Ruan et al., 1998](#)) and female ([Brucato et al., 1992](#)) rats. The current level used for baseline stimulation during the PP-DG LTP experiment was the current producing a PS on the first (P1) of the two evoked paired responses that was \sim 50% of maximum PS amplitude achieved in the I-O current curve. These current-paired pulse procedures were performed again at the termination of the LTP recording procedures to determine the effect of LTP on current intensity analysis and paired pulse facilitation and inhibition.

I-O current E-S coupling analysis was performed by averaging the three first pulse PS and EPSP slope measurements at each current level in the pre-LTP and post-LTP curves. These were then contrasted across a range of EPSP measures (low current, smaller EPSP slope; through higher current and larger measures of the synaptic response).

Measures are presented as absolute values (mV/ms and mV for slope and PS, respectively) and normalized to the largest, average PS and EPSP slope from the pre-LTP current curve ([Walling and Harley, 2004](#)).

Paired pulse ratios (PPR, pulse2/pulse1; P2/P1) were calculated for the PS amplitude at each ISI, and at each current intensity level. For presentation, the P2/P1 ratio from the stimulation intensity used for the LTP experiment (baseline; \sim 50% maximal PS) was contrasted with P2/P1 ratio at the same current intensity after LTP (post-LTP). For PPR current intensity analyses ([Extended Data Fig. 5-1](#)) outlier PPR values, in which the population spike amplitude on the second pulse was >3 SDs above normal, PPR values were capped to $+2$ SD of the highest PPR value for the animal at the respective ISIs. This treatment was applied to 4/360 PPR values in the six- to eight-

month-old male rats and 3/360 values in 18- to 20-month-old female rats.

Moderate strength burst long-term potentiation (LTP) stimulation

After completion of the initial I-O and paired pulse curves, a tetanic LTP protocol was tested. This protocol of the three tested by [Straube and Frey \(2003\)](#), the moderate Protocol B, produced robust, β -adrenergic receptor, and protein synthesis-dependent, LTP of the PS variable in behaving male Wistar rats. The PP was stimulated at 0.1 Hz with a monophasic 0.2-ms pulse and after 30 min of stable baseline evoked responses, monophasic tetanic stimulation at 200 Hz was applied, consisting of 20 trains of 15 pulses (0.25-ms width) with 10-s interburst intervals. Following the tetanic stimulation, the PP-DG evoked responses were followed for 90 min recording (0.1 Hz) followed by the second I-O and paired pulse analysis. Conventional absolute and normalized baseline and post-tetanic EPSP slope and PS measures are presented in Extended Data [Figure 2-1](#).

Assessment of the synaptic contribution to PS plasticity in adult and aged male and female rats

To assess the contribution of synaptic input on enduring PS potentiation, absolute measures of the EPSP slope and PS were arranged in 30-min bins and the EPSP slope measure of the first post-tetanic 30-min bin (0–30 min) was correlated (Pearson) with the three 30-min post-tetanic PS measures (0–30, 31–60, and 61–90 min post-LTP). For contrast, the EPSP slope and PS measures are also presented from the 30-min baseline period to illustrate pretetanus correlations.

Analysis of indexed NMDA receptor current during moderate strength tetanic LTP. To assess differences in NMDA receptor activation during tetanic in adult and aged adult male and female rats, total area under the curve (AUC) was measured first for each pulse within a 15 pulse stimulation train for each of the 20 burst stimulations, beginning at ~10 ms after the first pulse, a period determined previously to constitute NMDA receptor activation ([Racine et al., 1991](#); [Maren et al., 1992](#)). Total NMDA AUC was also summed across all 20 trains and also contrasted with the post-tetanic PS potentiation (see Extended Data [Fig. 4-1](#)).

Statistical analysis

With the exception of correlative variables, data were analyzed using multifactor analysis of variance (baseline EPSP slope and PS) or mixed design analysis of variance (age, sex, variable). Tukey's HSD test was used for *post hoc* assessments. All analyses were performed using Statistica v13.5 (StatSoft).

Results

Female rats show greater intrinsic granule cell excitability than males both pre-LTP and post-LTP. In adult male rats the LTP protocol induces an increase in excitability but does not induce an excitability increase in female rats

The involvement of intrinsic excitability in MPP LTP in adult male rats is consistent with the findings of [Lopez-](#)

[Rojas \(2016\)](#). The greater normalized LTP in adult male than adult female rats ([Fig. 2](#); Extended Data [Fig. 2-1C](#)) replicates [Maren \(Maren et al., 1994; Maren, 1995\)](#). Higher levels of excitability in the MPP circuit of female rats than male rats are seen in datasets from earlier studies ([Kehoe and Bronzino, 1999](#); [Zitman and Richter-Levin, 2013](#); [Safari et al., 2021](#); raw data shared by [Safari et al., 2021](#)), but see [Elmarzouki et al. \(2014\)](#) for counter example.

Adult (five- to nine-month) female rats have a leftward shift in E-S coupling relative to same age males, however PS increases in adult females appears to reach a ceiling effect ([Fig. 2A,E](#)), while absolute measures of PS amplitude in males are ~40–50% of female PS values in both adult and aged adult rats. Aged males and females have a similar E-S coupling EPSP slopes while aged female PS values increased post-LTP from pre-LTP values, a characteristic not observed in aged male rats. This argues that higher female intrinsic excitability, while possibly diminished in aged females from that of five- to nine-month female values, is still present.

Increases in the population spike post-LTP in female rats are most predicted by early (first 30 min post-LTP) increases in EPSP slope

In female rats, the PS-LTP following tetani is predicted by the EPSP slope increase occurring in the first 30 min post-tetani ([Fig. 3](#)). This post-tetani EPSP-spike correlation is not significant for male rats. When later post-LTP EPSP slope epochs (30–60 and 60–90 min) were examined to contrast with the results of the early EPSP changes, the EPSP slope measures were again, most predictive of PS values in female but not male rats of either age, however the latest (60–90 min) epoch in five- to nine-month female rats no longer reached significance indicating the early EPSP changes may be most predictive of PS amplitude changes over time; see Extended Data [Figure 3-1](#). Aged males also fail to show significant normalized slope or spike increases following tetani (see [Fig. 2](#); Extended Data [Fig. 2-1](#)). These outcomes argue that of the two MPP plasticity mechanisms identified by [Bliss and Lomo \(1973\)](#), an increase in synaptic size drives an increase in population spike in dentate gyrus of female rats, while in adult male rats plasticity depends on an increase in granule cell dendritic excitability as shown by [Lopez-Rojas \(2016\)](#). All groups showed a decrease in spike latency following tetani (Extended Data [Fig. 2-1](#)). This outcome is consistent with [Bliss and Lomo's](#) report that spike latency decrease was the most consistent response to LTP protocols ([Bliss and Lomo, 1973](#)). In a new study of MPP EPSP slope potentiation, [Amani et al. \(2021\)](#) report a decline in normalized EPSP potentiation beginning as early as eight months in male rats. The present failure of older males to exhibit EPSP potentiation corroborates their finding.

NMDA burst activation was stronger in adult males than in adult females or aged males

Using [Maren's](#) approach ([Maren et al., 1992](#)) of examining postburst depolarization to evaluate NMDA activation by tetani, we found the first three trains provided evidence of greater adult male (five to nine months) NMDA burst

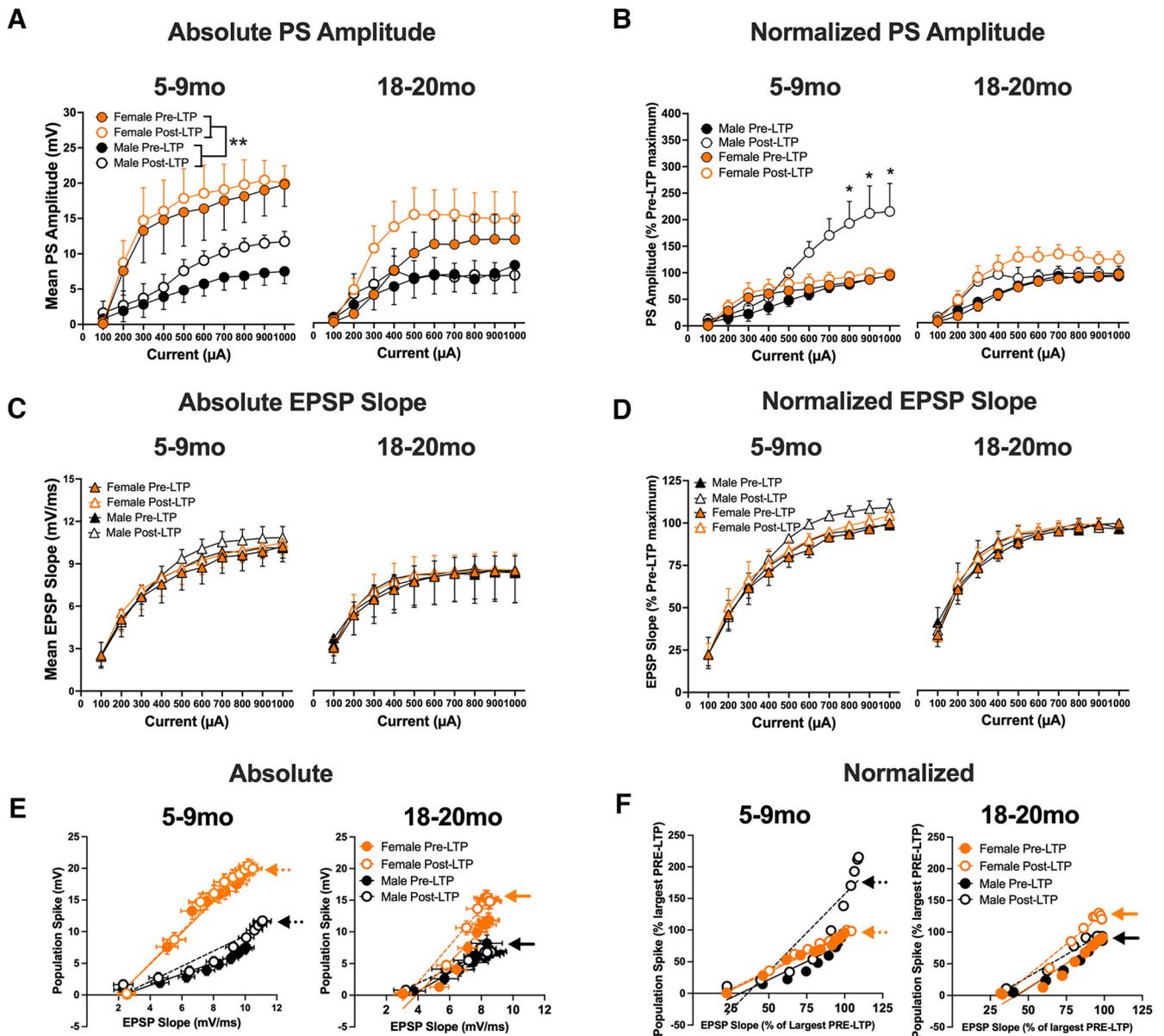


Figure 2. The effects of a moderate strength tetanic LTP protocol on PS and EPSP slope current intensity curves (input-output, I-O) and E-S coupling of the medial perforant path input to dentate gyrus in adult and aged adult, male and female rats. **A**, Absolute PS I-O values for adult and aged adult male and female rats pre-LTP and post-LTP. A main effect of sex was revealed ($F_{(1,23)} = 7.35$; $p = 0.01$). **B**, A significant sex, age, current, LTP interaction was found when PS values were normalized ($F_{(9,207)} = 3.27$; $p = 0.0009$). **C**, Absolute EPSP slope I-O analysis. **D**, Normalized EPSP slope I-O analysis. **E**, E-S coupling. Mean absolute EPSP slope (x-axis) and PS measures (y-axis) plotted at each current intensity (100–1000 μ A). EPSP and PS values in adult (5–9 months) rats indicate a leftward shift in E-S coupling values in female rats, and values indicative of a ceiling effect post-LTP (orange dashed arrow). In male rats at both ages PS values are ~40–50% of female PS values (black vs orange arrows). **F**, Relative E-S plot with data normalized to the largest average PS and EPSP slope values from the Pre-LTP I-O curve. Aged female rats expressed larger PS increases post-LTP but retained similar pre-LTP and post-LTP EPSP slope values, suggesting higher levels of intrinsic excitability support post-tetanic potentiation of the MPP input to DG (solid orange arrow). Aged male rats failed to express enduring potentiation of either EPSP slope or PS measures. Data represent means \pm SEM; however, error bars in **F** cannot not be observed because of the small levels of variability. * $p < 0.05$, ** $p < 0.01$. Sample waveforms and electrode placements, and conventional temporal LTP analyses of EPSP Slope and PS variables (absolute and normalized) are provided in Extended Data Figure 2-1.

depolarization than that seen in female rats of the same age (Fig. 4). Consistent with this finding, Maren et al. (1992) reported larger NMDA burst depolarization in adult males than adult females. NMDA burst depolarization here decreased successive trains across the sexes, in

both age groups. The outcome suggests NMDA induction is weakest in adult females, but not aged females. Effects of total NMDA AUC for the 15 pulses across the 20 trains was more predictive of PS potentiation in aged rats (see Extended Data Fig. 4-1).

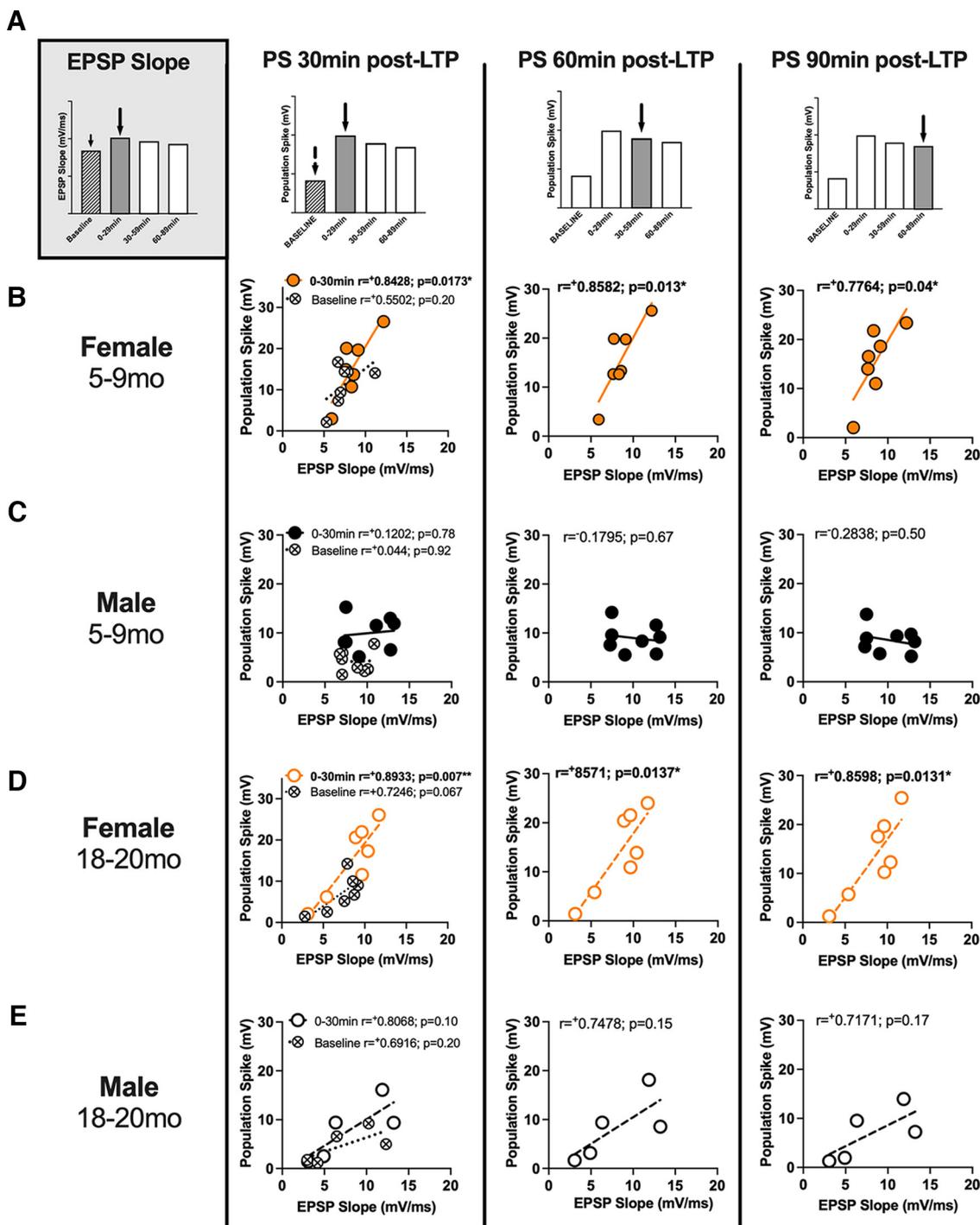


Figure 3. The effects of a moderate strength tetanic LTP protocol on the perforant path-dentate gyrus evoked population spike in young (5–9 months) and old (18–20 months) urethane-anesthetized female rats, correlate with early (0–30 min post-LTP) EPSP slope potentiation. **A**, Absolute EPSP slope and PS values were organized into 30-min bins (baseline, 0–30, 30–60, 60–90 min post-LTP). EPSP slope values for the baseline period, and 0–30 min post-LTP (early increases) were plotted against the baseline and the 0- to 90-min PS binned values. Baseline correlations indicate that EPSP slope is not significantly correlated with PS values in any sex, or age group (crossed circles, **B–E**); however, post-LTP EPSP slope values are predictive of increases in PS post-LTP in female rats (adult and aged; see bolded correlations in **B** and **D**). This effect was not observed in adult, or aged adult male rats (**C** and **E**). Additional comparisons of EPSP slope and PS measures at matched time bins (30–60 and 60–90 min) are provided in Extended Data Figure 3-1.

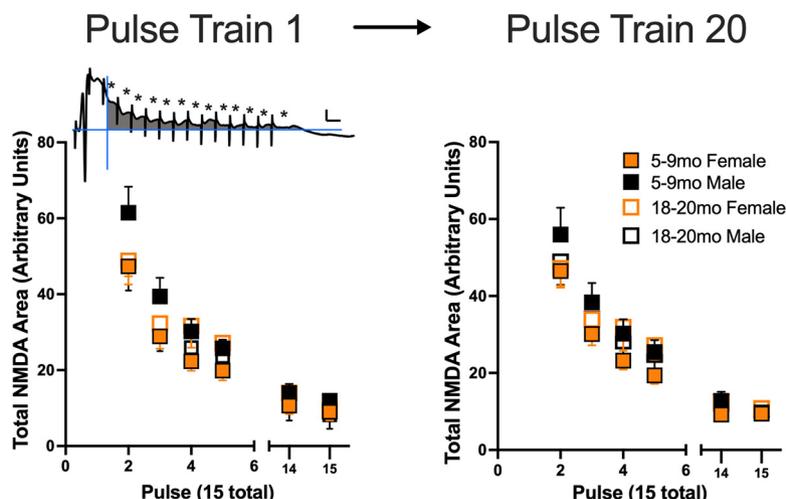


Figure 4. Assessment of the total NMDA receptor depolarization (Maren et al., 1992) for the first three trains during tetanic stimulation. The male adult rats (5–9 months, left panel) had a larger NMDA-dependent component than adult female rats ($F_{(1,13)} = 5.1936$; $p = 0.04$). In aged adult rats (right panel), there were no sex-dependent differences observed. In both age groups, there was a significant effect of trial with successive trains decreasing in the total NMDA-dependent response to the tetanic stimulation rats (adult, $F_{(2,26)} = 4.1925$; $p = 0.02$; aged adult, $F_{(2,20)} = 11.567$; $p = 0.0005$). Scale is 4 mV/5 ms. Total NMDA receptor contribution during moderate tetanic LTP stimulation correlated with PS increase in 30-min bins in adult and aged male and female rats (see Extended Data Fig. 4-1).

Paired pulse data revealed less GABA-B inhibitory modulation in female than male rats

Paired pulse inhibition probed at a 120-ms interval revealed the expected inhibition in male rats when the P1/P2 ratio was summed over all currents (~50% inhibition; see Fig. 5). Females, however, did not show evidence of 120-ms ISI inhibition with their P2/P1 ratios averaging close to or above 1.0. Reduced GABA-B-mediated inhibition, indexed by this probe, may contribute to greater female intrinsic excitability. Canning and Leung (2000) demonstrated that *in vivo* granule cell excitability is controlled by GABA-B-mediated inhibition. See Extended Data Figure 5-1 for full profile of input-output current curve paired pulse results.

Discussion

The present experiments provide evidence for both sex-related and age-related differences in MPP plasticity in rat dentate gyrus. The sex difference in intrinsic excitability requires replication. While re-examining control data in earlier studies with other objectives provides some support for our findings, only one other laboratory has specifically addressed this variable. Maren’s studies did find greater normalized spike potentiation among Sprague Dawley males under two kinds of anesthesia, but they specifically matched male and female rats for spike size potentially eliminating the differences in absolute spike/current relationships seen here.

What sex differences might account for these outcomes? Erwin et al. (2020) demonstrate that a subtype of mature granule cell, the PENK-expressing granule cell is more excitable than other subtypes and because of its greater excitability is preferentially recruited to spatial maps. They argue that this subtype dominates recruitment in hippocampally-dependent behaviors and supports

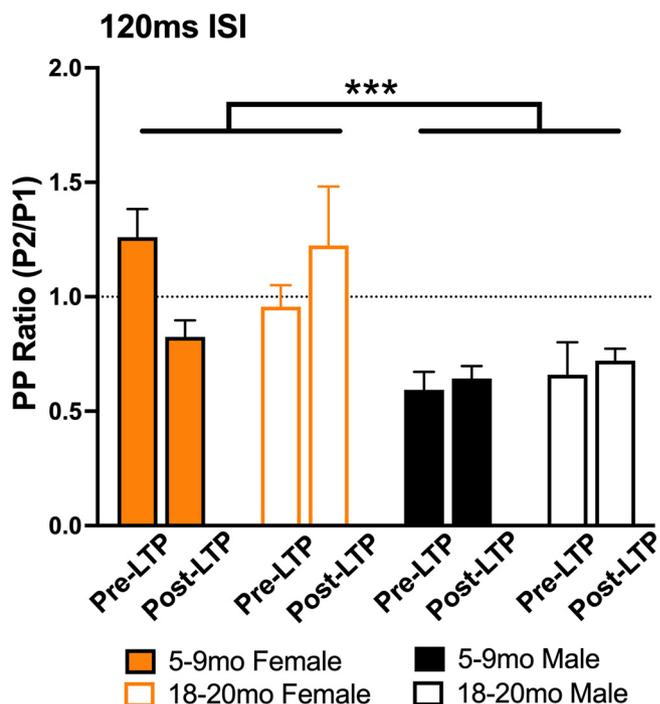


Figure 5. Paired pulse inhibition (120-ms ISI) and facilitation of the PP-DG evoked population spike in young and old male and female rats. P2/P1 ratio for GABA-B dependent PPI (in 5- to 9- and 18- to 20-month male and female rats at current levels used for baseline recordings; 50% maximal PS on P1), and same current post-LTP. Female rats demonstrated less PPI compared with male rats. There was a significant main effect of sex ($F_{(1,23)} = 17.49$; $p = 0.0003$). Data represent means \pm SEM, $***p < 0.001$. See Extended Data Figure 5-1 for full description of PP input-output profiles.

sparse spatial representations in male mice. The critical role of intrinsic excitability in determining dentate gyrus MPP output was also recently highlighted by Zhang et al. (2020). Oulé et al. (2021) identified the potassium channels responsible for dendritic intrinsic excitability in male mice as the Kv4.2 subtype and revealed that these channels are critical for spatial pattern separation.

In a new study identifying PENK-expressing granule cells in both male and female rats, Johnson et al. (2021) demonstrate that female dorsal dentate gyrus contains a significantly larger proportion of PENK-expressing granule cells than male dentate gyrus. The higher PENK-cell proportion in females may support their greater intrinsic excitability. Based on the data from mice, MPP-supported spatial and nonspatial representations might be predicted to be less diffusely distributed, and therefore possibly more robust, in female rats than males (see, for example, Zhvania et al., 2021; Olave et al., 2022; O'Leary et al., 2022). Experimental evidence with respect to place field and episodic representation in female versus male rats is needed. In an evolutionary context (Sherry et al., 1992) spatial map demands appear different for male and female rats. Male *Rattus norvegicus* rats have spatial territories an order of magnitude larger than females (Oyedele et al., 2015) and may require better spatial resolution for those territories.

The role of aging in dentate gyrus plasticity has been more extensively studied. A selective decrease in MPP synapses on granule cells dendrites (3 vs 28 months) was reported in 1976 (Bondareff and Geinisman, 1976). Barnes (1979) comparing behavioral and LTP measures in 10- to 16- versus 28- to 34-month-old male rats found repeated trains produced enduring EPSP slope potentiation at 10–16 months but a declining potentiation in the senescent rats. Barnes et al. (2000) report reduced NMDA currents and a higher threshold for EPSP LTP in senescent rats, which were memory-impaired (see also Yang et al., 2008). Their middle-aged group (9–10 months), are similar to the adult rats here and appeared intermediate between young and senescent rats in depolarization needed for EPSP slope potentiation. In the Barnes and colleagues study, the NMDA currents in female rats were not examined and warrant further investigation. Arc-active granule cells with spatial exploration decline across young, middle and aged rats (Small et al., 2004).

The state of estrus or levels of circulating hormones were not monitored in this study. Gould et al. (1990) examined dendritic spine density changes in the hippocampus across the estrus cycle and found density changes in CA1, but did not find that estrus affected spine density in dentate gyrus granule cells. Kehoe and Bronzino (1999) similarly did not find differences in dentate gyrus LTP across the estrus cycle in awake female rats (three-month-old rats). Together, this suggests the state of estrus in the five- to nine-month female rats was not a significant influence on plasticity. It would be presumed that the 18- to 20-month female rats had low levels of brain and circulating estrogen, however aged females still expressed higher levels of PS plasticity compared with aged male rats. Influences of estrogen across the lifespan may

still account for these differences potentially providing a plasticity “reserve” in aged females. Sex-dependent differences in neurogenesis in dentate gyrus during aging may similarly explain the changes in dentate gyrus plasticity (Yagi et al., 2020; Hodges et al., 2022). Given that MPP-dentate gyrus plasticity in aged females is remarkably spared compared with age-matched male rats in the present study, investigations into the possible neuroprotective role of estrogen, and influence on neurogenesis during aging should be comprehensively examined.

Multiple laboratories (Rapp and Gallagher, 1996; Lubec et al., 2019) have demonstrated both memory-impaired and memory-unimpaired aged male rats are seen when probed on hippocampally-dependent tasks, thus variability in initiating and maintaining plasticity with age is likely. Informal examples of age-related and sex-related heterogeneity of dentate gyrus plasticity can be observed in variability measures in long-term plasticity (see Extended Data Fig. 2-1) and short-term plasticity (see aged female rats in Extended Data Fig. 5-1 at low stimulation levels). Future studies could further examine heterogeneity of plasticity measures related to age and sex and its possible relationship with behavior. Understanding the mechanistic underpinning of that variability will be useful for cognitive anti-aging strategies. Lubec et al. (2019) working with Sprague Dawley rats aged 22–24 months and comparing them to six-month rats identified proteomic changes that occurred differentially in aged impaired and unimpaired rats. Impaired rats were deficient in pathways related to energy metabolism and potassium ion regulation. They found unimpaired rats differed from the general population as early as six months of age corroborating the Amani et al. (2021) assertion that decreases in MPP plasticity are an early harbinger of aging. Lubec et al. (2021) later found that increasing dopamine in aged male Sprague Dawley rats with both intermediate and severe behavioral impairments on hippocampally-dependent tasks restored behavior to that of young rats and restored spine numbers on granule cells to young levels. Both spatial behavior and cognitive flexibility were improved.

The present experiments suggest MPP-related aging changes are likely to differ among males and females with males being more vulnerable to aging-related disruption of plasticity and likely to show greater impairment on hippocampally-dependent tasks. This hypothesis remains to be explored.

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