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Disrupting Jagged1–Notch signaling impairs spatial memory formation in adult mice

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ABSTRACT

It is well-known that Notch signaling plays a critical role in brain development and growing evidence implicates this signaling pathway in adult synaptic plasticity and memory formation. The Notch1 receptor is activated by two subclasses of ligands, Delta-like (including Dll1 and Dll4) and Jagged (including Jag1 and Jag2). Ligand-induced Notch1 receptor signaling is modulated by a family of Fringe proteins, including Lunatic fringe (Lfng). Although Dll1, Jag1 and Lfng are critical regulators of Notch signaling, their relative contribution to memory formation in the adult brain is unknown. To investigate the roles of these important components of Notch signaling in memory formation, we examined spatial and fear memory formation in adult mice with reduced expression of Dll1, Jag1, Lfng and Dll1 plus Lfng. We also examined motor activity, anxiety-like behavior and sensorimotor gating using the acoustic startle response in these mice. Of the lines of mutant mice tested, we found that only mice with reduced Jag1 expression (mice heterozygous for a null mutation in Jag1, *Jag1*$^{+/-}$) showed a selective impairment in spatial memory formation. Importantly, all other behavior including open field activity, conditioned fear memory (both context and discrete cue), acoustic startle response and prepulse inhibition, was normal in this line of mice. These results provide the first *in vivo* evidence that Jag1–Notch signaling is critical for memory formation in the adult brain.

1. Introduction

The Notch pathway is a highly-conserved ubiquitous signaling system which plays a fundamental and well-studied role in cell–cell communication [reviewed in (Artavanis-Tsakonas, Rand, & Lake, 1999; Guruharsha, Kankel, & Artavanis-Tsakonas, 2012; Kopan, 2012)]. *Notch* genes encode single-pass transmembrane receptor proteins. *Drosophila* have a single *Notch* gene (del Amo et al., 1993), *C. elegans* have two *Notch* genes [*LIN-12*, *GLP-1*]) (Austin & Kimble, 1989), while mammals have four [*Notch*1–4] (del Amo et al., 1993; Lardelli, Dahlstrand, & Lendahl, 1994; Uyttendaele et al., 1996). In mammals, there are two subclasses of Notch ligands, Delta-like (Dll1 and Dll4, referred to as Delta in *Drosophila*) and Jagged (Jag1 and Jag2, referred to as Serrate in *Drosophila*) [reviewed in (Bray, 2006)]. The ability of these ligands to activate Notch signaling is modulated by glycosylation mediated, in part, by a family of sugar transferases termed Fringe proteins (Brückner, Perez, Clausen, & Cohen, 2000; Fleming, Gu, & Hukriede, 1997; Klein & Arias, 1998; Moloney et al., 2000a,b; Panin, Papayannopoulos, Wilson, & Irvine, 1997). In mammals, there are three *Fringe* genes: *Lunatic fringe* [*Lfng*], *Manic fringe* [*Mfng*] and *Radical fringe* [*Rfng*] (Cohen et al., 1997; Johnston et al., 1997). Lfng can both enhance Notch1 signaling induced by Dll1 and suppress Notch1 signaling induced by Jag1 (Hicks et al., 2000).

Canonical Notch signaling is initiated by a Notch ligand, expressed on the surface of one cell, binding to the extracellular domain of a Notch receptor that is located on the surface of a neighboring cell. In this way, Notch signaling is somewhat unique in that signaling is restricted to neighboring cells. Once activated, the Notch receptor undergoes proteolytic cleavage and the soluble (and active) Notch intracellular domain (NICD) is released. NICD then translocates to the nucleus where it regulates target gene expression by associating with the central DNA binding transcription factor RBP-J (Kao et al., 1998; Schroeter, Kisslinger, & Kopan, 1998; Struhl & Adachi, 1998). RBP-J normally represses gene

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expression, but when bound to NICD, gene expression is activated (Jarriault et al., 1995; Kato et al., 1997).

Although traditionally studied in cell fate specification during development, the Notch signaling pathway is increasingly recognized to play an important role in the adult nervous system (Yoon & Gaiano, 2005). Notch is expressed by neurons in the adult mouse brain where it is present at particularly high levels in the hippocampus (Berezovska, Xia, & Hyman, 1998). In addition, the Notch ligands, Jag1 and Dll1, are also expressed in the hippocampus (Breunig, Silbereis, Vaccarino, Sestan, & Rakic, 2007; Stump et al., 2002). Several studies implicate Notch signaling in adult synaptic plasticity, learning, and memory [reviewed in (Costa, Drew, & Silva, 2005)]. For instance, increasing Notch function enhances long-term memory formation, whereas disrupting Notch inhibits memory formation in *Drosophila* across several paradigms (Ge et al., 2004; Matsuno, Horiuchi, Tully, & Saitoe, 2009; Presente, Boyles, Serway, de Belle, & Andres, 2004). Consistent with this, long-term potentiation (LTP), argued to be a cellular correlate of memory formation, is impaired in mice with reduced Notch1 protein in the hippocampus [produced by expressing antisense directed against *Notch1* mRNA] (Wang et al., 2004). Importantly, this impairment is reversed by application of the ligand Jag1 (Wang et al., 2004). Furthermore, although homozygous deletion of *Notch1* is embryonically lethal (Swiatek, Lindsell, del Amo, Weinmaster, & Gridley, 1994), heterozygous *Notch1*$^{+/-}$ mice develop normally, but show specific deficits in spatial memory formation (Costa, Honjo, & Silva, 2003). This finding was recently confirmed and extended in mutant mice in which *Notch1* is conditionally knocked out in adult forebrain neurons (Alberi et al., 2011).

These findings suggest that Notch1 signaling regulates memory formation in adult mice. However, the potential roles of the different Notch1 ligands and Fringe proteins are unknown. Here we examined learning and memory in several lines of mice with reduced levels of the Notch1 ligands Dll1 and Jag1, as well as a critical modulator of Notch receptor-ligand affinity, Lfng. In each case, we used mice that were heterozygous for a null mutation and found that reduced expression of Jag1 (but not Dll1 or Lfng) produced deficits in spatial memory formation while sparing fear memory formation. Importantly, Jag1$^{+/-}$ mice showed normal motor activity, anxiety-like behavior, and sensorimotor gating, suggesting that the spatial memory impairment was specific.

2. Materials and methods

2.1. Mice

All experimental procedures were conducted in accordance with Canadian Council on Animal Care (CCAC) guidelines and approved by the Animal Care Committee at the Hospital for Sick Children. Adult male and female C57Bl/6NTac mice (3–4 months old) were used for all experiments. Mice were housed 3–5 animals per cage on a 12 h light/dark cycle and provided with food and water *ad libitum*.

All lines of mice were heterozygous for a null mutation encoding each gene of interest. Generation of a *Dll1*$^{+/-}$ was described previously (Hrabe de Angelis, McIntyre II, & Gossler, 1997). *Lfng* loss-of-function allele (*Lfng*$^{-}$) was generated by deletion of exon 2 (Xu et al., 2010). Double heterozygous mice (*Dll1*$^{+/-}$/*Lfng*$^{+/-}$) were generated by crossing *Dll1*$^{+/-}$ and *Lfng*$^{+/-}$ single heterozygous mice. *Jag1*$^{-}$ allele was produced by replacement of a portion of the extracellular domain (the last 3 EGFL repeats and the CR domain) as well as the entire intracellular domain with a transmembrane domain linked to *β-geo* (Xu et al., 2012). For all experiments, mice heterozygous for the desired mutation (*Dll1*$^{+/-}$, *Lfng*$^{+/-}$, *Dll1*$^{+/-}$/*Lfng*$^{+/-}$, *Jag1*$^{+/-}$) were generated from wild-type (WT) X heterozygous (HET) parents, and their respective WT littermates were used as controls. Mice were handled for 7 days (2–4 min) prior to the start of behavioral testing. Multiple behavioral tests were conducted on all mice in the following test order: open field, Morris water maze, contextual and cued fear conditioning, acoustic startle response (habituation), prepulse inhibition of the acoustic startle reflex and threshold for an acoustic stimulus to elicit a startle reflex.

2.2. Morris water maze

The Morris water maze was used to assess spatial memory formation (Morris, 1984). A circular tank (diameter 120 cm, depth 50 cm) was filled with warm water (28 ± 1 °C, depth 40 cm) made opaque by the addition of non-toxic tempura paint. A circular escape platform (diameter 10 cm) was submerged 0.5 cm below the water surface. The platform was located in a fixed position throughout training. Mice were trained to locate the platform using extra-maze cues placed 1 m from the pool perimeter.

Mice were trained with one of two training protocols. In the intense training protocol, mice received 2 blocks of 3 trials per day, for 6 training days. In the weak training protocol, mice received 3 blocks of 4 trials per day for 3 training days. Trials were initiated by placing the mouse into the pool facing the wall at one of four randomly assigned start positions. Mice were allowed to swim until they found the platform, or until 60 s elapsed. Mice that failed to find the platform within 60 s were guided to the platform. After each training trial, the mouse remained on the platform for 15 s. A probe test (during which the platform was removed and mice placed into the pool for 60 s) was conducted at the end of training to assess spatial memory formation. Mice that formed a memory of the spatial location of the platform tended to swim in the area of the pool in which the platform was previously located. The probe test for the intense training protocol was performed on day 7, 24 h after completion of training. The probe test for the weak training protocol was conducted 60 min after the final training trial. A visible version of the water maze task was performed on one line of mice. In this version, the platform was marked with a local cue throughout the 3-d training protocol.

The swimming paths were acquired by an overhead video camera and analyzed using an automated tracking system (Actimetrics, Wilmette, IL). Escape latency and swim speed during training were recorded for each mouse. The percentage of time mice spent in the Target Zone (a 20 cm radius zone centered on the former platform location) versus the average of three other equivalent zones of the pool (but located in the other 3 quadrants, Other Zone) in the probe tests was quantified and used as our index of spatial memory formation.

2.3. Contextual and cued fear conditioning

During training, mice were placed in a Med Associates (St. Albans, VT) chamber (24 × 30 × 21 cm) located in a soundproof room and allowed to explore the environment for 2 min. Mice were presented with a tone (85 dB, 2800 Hz) for 30 s, that co-terminated with a foot shock [0.5 mA, 1 s]. 24 h after training, context fear memory was assessed by placing mice in the training context and freezing was assessed for 5 min. 24 h later, discrete cue fear memory was assessed by placing mice in a novel context. 2 min later the tone was played for 3 min. Percentage of time mice spent freezing (lack of movement except respiration (Fanselow, 1980)) was recorded by a video camera, assessed using FreezeFrame and FreezeView (ActiMetrics) software, and used as our index of fear memory.

2.4. Open field

The open field test was used to measure overall motor activity and anxiety-like behavior. Each mouse was placed in the middle of a Plexiglas box (45 × 45 × 20 cm), evenly lit from above, and allowed to explore the environment for 15 min. The position of the mouse was recorded by an overhead video camera and analyzed using LimeLight 2 software (ActiMetrics). This software divided the open field into 3 zones; Zone 1 = the outermost perimeter of the box, Zone 2 = the middle zone, and Zone 3 = center zone. The overall distance travelled and percentage of time spent in each of the three zones was assessed.

2.5. Auditory startle response

The acoustic startle reflex was tested using an SR-LAB startle testing system (San Diego Instruments, CA, USA). Mice were placed in a Plexiglas testing cylinder (3.2 cm internal diameter). Acoustic startle stimuli and prepulse stimuli were delivered via a high-frequency speaker, placed 15 cm from the testing cylinder. Background white noise was generated by a standard speaker. The testing cylinder was mounted on a sensor platform. A piezoelectric accelerometer, attached to the base of the sensor platform, detected and transduced cage movements that were then digitized by and stored in a computer. Startle amplitude was taken as the maximal response that occurred in 100 ms after presentation of the startle stimulus. The sound levels for background noise and startle/prepulse stimuli were calibrated with a digital sound level meter. The speakers, testing cylinder and sensor platform were housed within a sound-attenuated chamber.

2.5.1. Habituation of the acoustic startle response

Mice were placed in the testing cylinder and 5 min later, presented with 80 startle pulses of 120 dB each (15 s interstimulus interval, ISI).

2.5.2. Threshold of the acoustic startle response

The following day, the startle threshold for each mouse was determined. Following a 5-min acclimation period, mice were presented with a total of 99 trials (15 s ISI). There were 11 trial types: no stimulus (NS), and 10 types of startle trials in which the intensity of the startle stimulus randomly varied from 75 to 120 dB (with 5 dB increments). The startle stimuli were 40 ms noise bursts with a rise/fall time of less than 1 ms. The 11 trial types (NS, startle stimuli) were presented in a pseudorandom order such that each trial type was presented once within a block of 11 trials. Startle threshold was defined as the minimal intensity at which responding was significantly greater than in the NS trials.

2.5.3. Prepulse inhibition of the acoustic startle response

Prepulse inhibition refers to the phenomenon in which a response to a strong startling stimulus is weakened or inhibited if preceded by a weaker stimulus. A reduction or inhibition of a startle response by a prepulse is thought to reflect the ability of an organism to temporarily change responding to changing circumstances. A disruption of prepulse inhibition is thought to reflect an inability to filter non-relevant stimuli.

24 h after determining the startle threshold, mice were tested for prepulse inhibition of the startle response. Following a 5-min acclimation period, mice were presented with 20 habituation trials (120 dB, ISI 15 s). In the prepulse inhibition phase, mice were presented with a total of 90 trials. Three prepulse intensities were tested: 70, 75 and 80 dB. Prepulse stimuli were 20 ms in duration with a rise/fall time of less than 1 ms. For each prepulse intensity, there were three types of trials: prepulse alone, prepulse + startle stimulus, and startle stimulus alone. In the prepulse + startle stimulus trial, onset of the prepulse preceded onset of the startle stimulus by 100 ms. All startle stimuli were presented in a pseudorandom sequence with the constraint that each stimulus intensity occur only once in each consecutive four-trial block. The percentage prepulse inhibition (%PPI) was calculated per mouse for each of the three prepulse conditions using the formula %PPI = [1 – (startle amplitude elicited by prepulse + startle stimulus)/(startle amplitude elicited by startle stimulus alone)] × 100. A high %PPI score indicates more sensorimotor gating.

2.6. Statistical analyses

Data were analyzed with 1 and 2-way analyses of variance (ANOVAs) using Statistica (Statsoft) software. For water maze, we analyzed escape latency during training and the time spent in Target versus Other Zones during water maze probe trials. For fear conditioning, we compared the time spent freezing in the context and to the tone. Newman–Keuls post hoc tests were performed as appropriate.

3. Results

3.1. Spatial memory formation is impaired in $Jag1^{+/-}$ mice

To assess spatial learning and memory in adult $Dll1^{+/-}$, $Lfng^{+/-}$, $Dll1^{+/-}/Lfng^{+/-}$ and $Jag1^{+/-}$ mice, we used the hidden platform version of the Morris water maze, a task critically dependent on hippocampal function (Morris, 1984). We compared performance of the mutant mice to their respective WT littermates. Mice were trained to find a hidden platform submerged below the water surface, located in a fixed position throughout training (6 days of training with 2 blocks of 3 trials/day). We examined spatial memory formation in a probe test conducted at the end of training during which the platform was removed from the pool.

3.1.1. Intense water maze training protocol (6 days)

3.1.1.1. Training. All groups, regardless of line (*Dll1*, *Lfng*, *Dll1*/*Lfng*, *Jag1*) or genotype (WT, HET), showed decreased latencies to find the platform over the course of training (Fig. 1, left panel). This interpretation was supported by the results of a series of ANOVAs conducted on each line of mice [with between-groups factor *Genotype* (HET, WT) and within-groups factor *Days* (6)] (Table S1). Briefly, in the *Dll1* line, both $Dll1^{+/-}$ mice (HET) and their WT littermate controls showed decreased latencies to find the platform over training days, although $Dll1^{+/-}$ mice showed longer escape latencies overall than WT mice (Fig. 1B). In the *Lfng* line, both HET and WT mice showed decreased escape latencies over training days that did not differ (Fig. 1C). A similar pattern of results was observed in the *Dll1*/*Lfng* and *Jag1* lines of mice; both $Dll1^{+/-}$/$Lfng^{+/-}$ and $Jag1^{+/-}$ mice showed a decrease in latency over days that did not differ from WT littermate mice (Fig. 1D and E). Therefore, in general, all mice, regardless of line or genotype, located the platform faster over training days (with the exception of $Dll1^{+/-}$ which showed a decrease in latency over days, but were slower than their WT littermates). Importantly, these various mutations in the Notch signaling pathway did not affect swim speed or thigmotaxis during training [no effect of *Genotype* (HET versus WT) in each line, data not shown].

3.1.1.2. Probe test. To assess spatial memory formation, we conducted a probe test (during which the platform was removed from the pool) 24 h after the final training trial. We quantified the amount of time mice spent searching in the target zone (20 cm radius, centered on the location of the platform during training; 11% of the total pool surface) versus the time spent in an average of

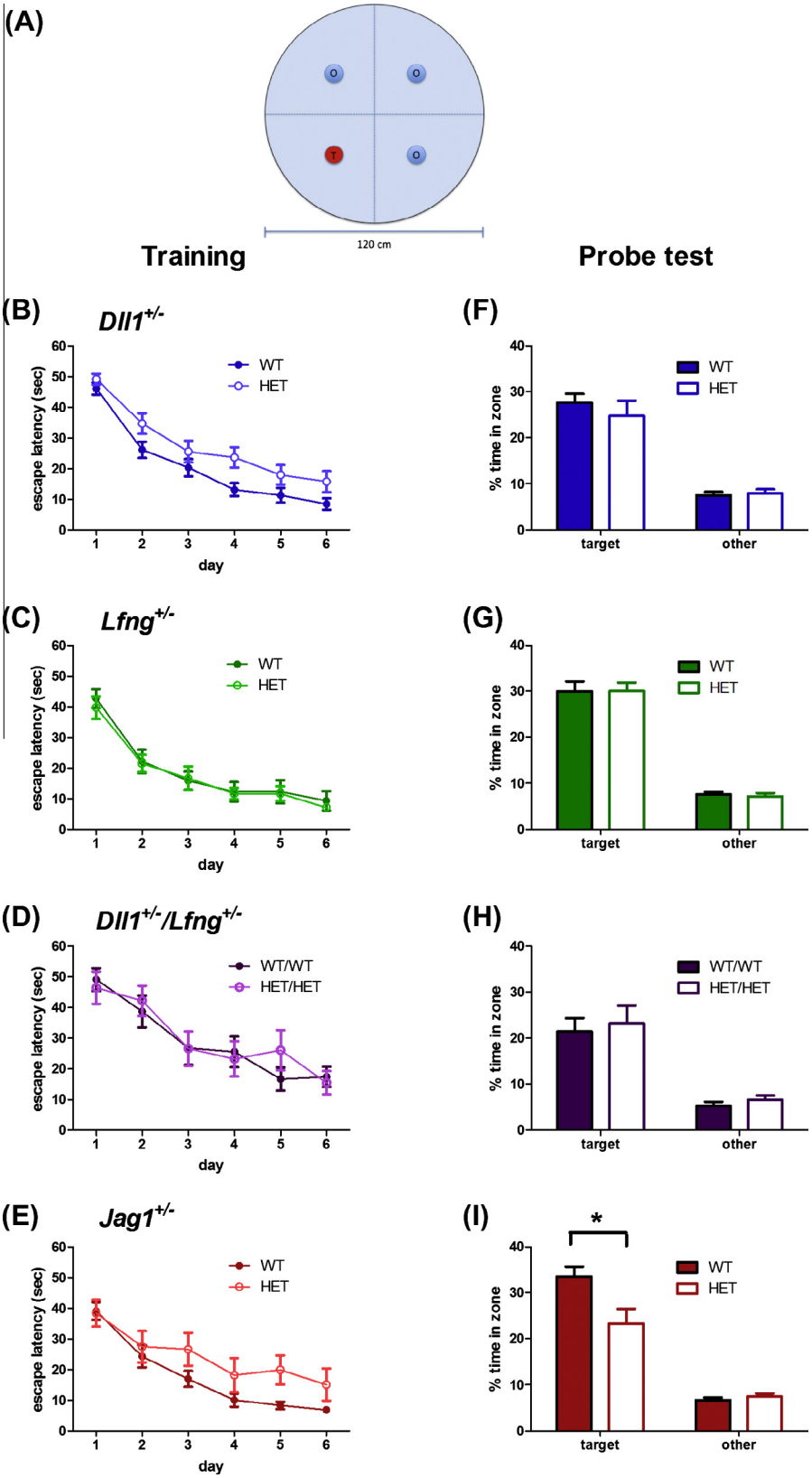

Fig. 1. Spatial memory formation is impaired in *Jag1*$^{+/-}$ mice only. (A) Schematic representation of the water maze pool showing Target (T) and Other (O) zones. (B–E) Escape latency during intense water maze training (6 days). (B) *Dll1*$^{+/-}$ mice ($n = 23$) required more time to reach the platform than their WT littermates ($n = 25$). There was no difference in escape latency between, (C) *Lfng*$^{+/-}$ ($n = 15$) and WT ($n = 14$), (D) *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ ($n = 8$) and WT/WT ($n = 11$), (E) *Jag1*$^{+/-}$ ($n = 11$) and WT ($n = 19$) mice. (F–I) Spatial memory formation as assessed in a probe trial. (F) *Dll1*$^{+/-}$, (G) *Lfng*$^{+/-}$ and (H) *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ mice performed similarly to their WT littermates in the probe trial, spending more time in the Target versus the Other zones of the pool (indicating robust spatial memory formation). (I) *Jag1*$^{+/-}$ mice spent less time in the Target zone than their WT controls, indicating a spatial memory deficit. Data represent mean ± SEM.$^{*}p < 0.05$.

three other equivalent zones located in different quadrants of the pool (see Fig. 1A). We conducted an ANOVA with a between-subjects factor of *Genotype* (WT, HET) and a within-subjects factor of *Zone* (Target, Other) for each line of mice (Table S1).

In general, most lines of mice (*Dll1*, *Lfng*, *Dll1/Lfng* mice), spent significantly more time in the Target zone compared to the Other zones of the pool, indicating formation of spatial memory. Furthermore, in these lines, there was no difference in the time spent in the Target zone between HET and WT mice (Fig. 1F–H). That is, although there was a significant effect of *Zone*, there was no significant effect of *Genotype* or *Zone X Genotype* interaction (see Table S1 for full statistical details). The exception to this general finding was *Jag1*$^{+/-}$ mice. *Jag1*$^{+/-}$ mice spent significantly less time in the Target zone than their WT littermates (Fig. 1I) (Significant *Genotype* X *Zone* interaction [$F(1,28) = 8.1$, $p < 0.05$], as well as significant main effects of *Zone* [$F(1,28) = 120.3$, $p < 0.001$] and *Genotype* [$F(1,28) = 6.4$, $p < 0.05$]). Post-hoc Newman–Keuls analysis of the significant interaction revealed that *Jag1*$^{+/-}$ mice spent significantly less time in the Target zone than their WT littermates ($p < 0.05$). These results suggest that although *Jag1*$^{+/-}$ mice formed a spatial memory, this memory was weaker than the memory formed by their WT littermate controls.

3.1.2. Weak water maze training protocol (3 days)

To further investigate the spatial memory deficit observed in *Jag1*$^{+/-}$ mice, we trained a separate cohort of mice using a weaker training protocol (2 blocks of 4 trials/day over 3 days). As with the intense training protocol, both *Jag1*$^{+/-}$ and WT mice showed a decrease in latency to find the platform over the course of training (Fig. 2A). However, *Jag1*$^{+/-}$ mice took significantly longer to reach the platform than their WT littermate controls (Fig. 2A). An ANOVA revealed significant effects of *Genotype* [$F(1,29) = 6.8$, $p < 0.05$] and *Day* [$F(2,58) = 124.1$, $p < 0.001$], but no significant *Genotype* by *Day* interaction [$F(2,58) = 0.5$, $p > 0.05$].

During the probe test, *Jag1*$^{+/-}$ mice spent less time in the Target zone compared to their WT littermates (significant *Genotype* by *Zone* interaction [$F(1,29) = 7.7$, $p < 0.05$], as well as significant main effects of *Genotype* [$F(1,29) = 8.0$, $p < 0.05$] and *Zone* [$F(1,29) = 49.3$, $p < 0.001$]. Newman–Keuls post hoc analysis conducted on the significant interaction showed that *Jag1*$^{+/-}$ mice spent significantly less time in the Target zone than WT mice ($p < 0.001$) (Fig. 2B). Importantly *Jag1*$^{+/-}$ mice showed intact performance in a visible platform version of the water maze (Fig. 2C, Table S1). Taken together, these findings indicate that reduced expression of Jag1 (but not Dll1 or Lfng) impaired spatial memory formation.

3.2. Fear memory formation is intact in all lines of mice

We next investigated the role of Notch signaling in different memory tasks by examining these mouse lines in cued and contextual fear conditioning. We trained mice in a single conditioning session in which a tone (conditioned stimulus, CS) was paired with a shock (unconditioned stimulus, US). 24 h later, mice were returned to the context in which they were previously shocked and the amount of time spent freezing was measured (context fear). Cued fear memory was assessed 24 h later when mice were placed in a novel context and the tone was presented. The amount of time spent freezing before and during the tone CS was measured (tone fear).

Across all lines of mice, both WT and HET showed equal and robust levels of freezing during both the context (Fig. 3A–D) and tone (Fig. 3E–H) fear memory tests. An ANOVA conducted on each line revealed no significant differences between genotypes (p's > 0.05, Table S2). Together, these results indicate that decreasing the levels of the Notch ligands or Fringe does not impact either contextual or cued fear conditioning.

3.3. Open field behavior is normal in *Dll1*$^{+/-}$, *Lfng*$^{+/-}$ and *Jag1*$^{+/-}$ mice

We next examined the effects of disrupting Notch signaling on overall locomotor activity and anxiety-like behavior by assessing the behavior of mice in a brightly-illuminated open field. First,

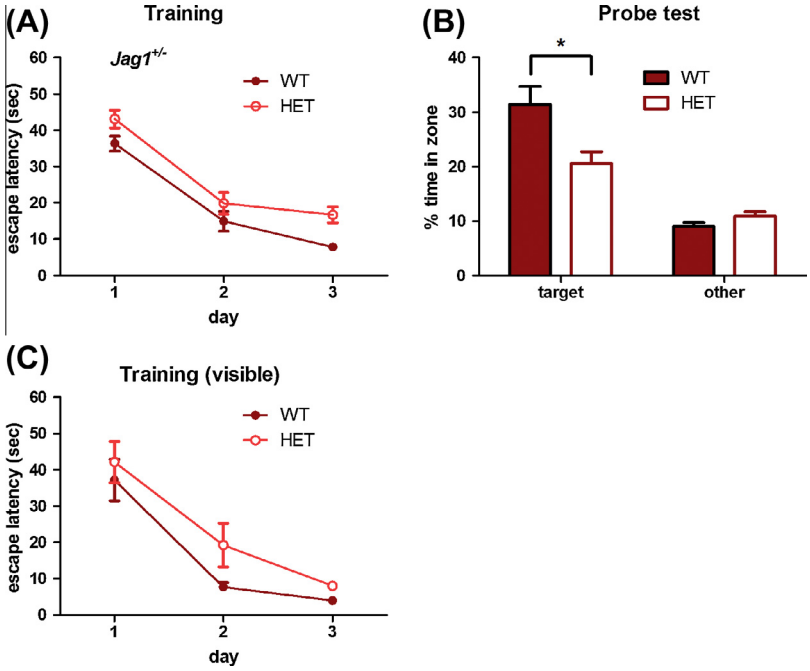

Fig. 2. Impaired spatial memory in *Jag1*$^{+/-}$ mice was confirmed using weak water maze training protocol. (A) Escape latency of *Jag1*$^{+/-}$ mice during weak water maze training (3 days). HET mice ($n = 17$) required more time to reach the platform than WT controls ($n = 14$). (B) In the probe trial, HET mice spent less time searching in Target (T) zone than WT mice, indicating a spatial memory deficit. (C) Escape latency of *Jag1*$^{+/-}$ mice trained in visible platform version of the water maze was not different in HET ($n = 5$) versus WT ($n = 8$) mice, indicating that the spatial memory deficit observed in *Jag1*$^{+/-}$ mice cannot be explained by a performance deficit. Data represent mean ± SEM. $^{*}p < 0.05$.

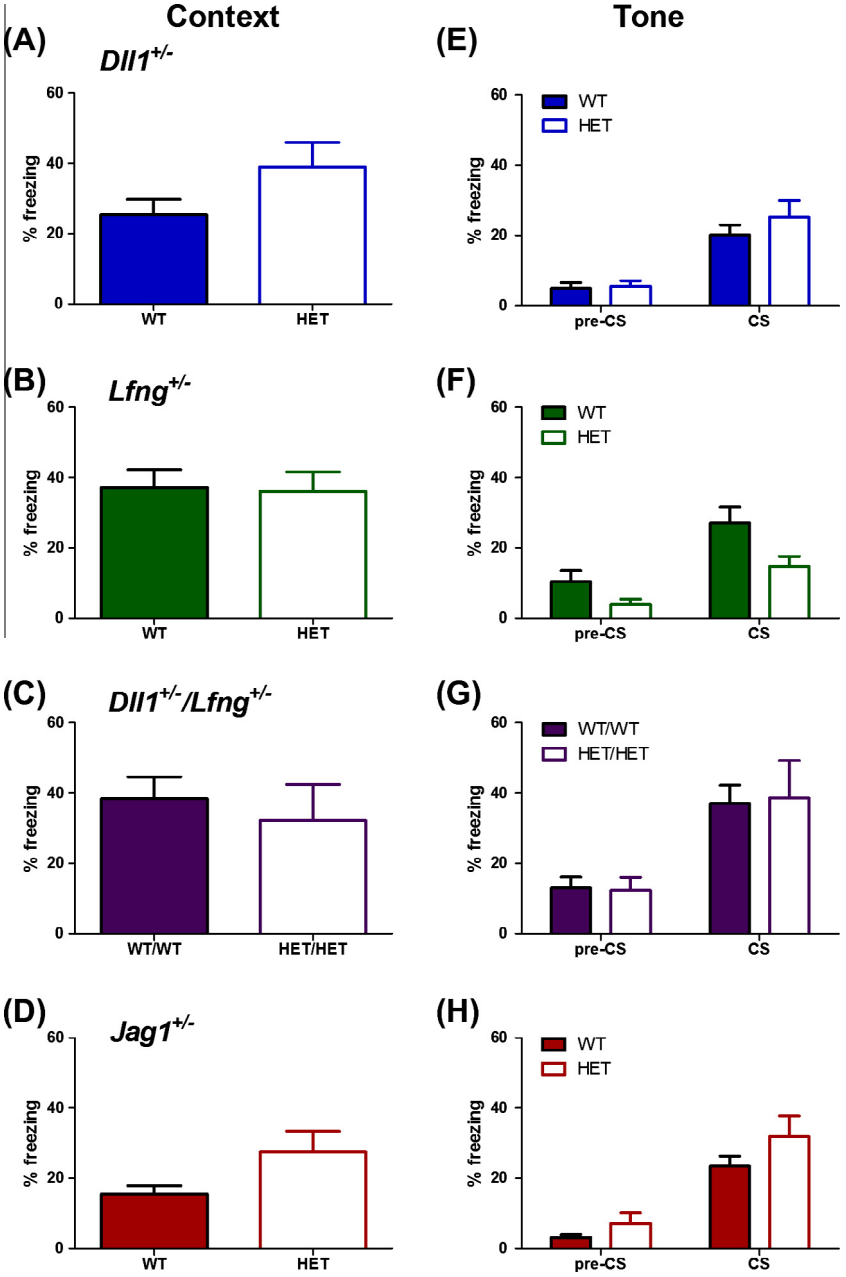

Fig. 3. Normal context and tone fear memory in all lines of Notch mutants. Freezing levels in context (A–D) and tone (CS) (E–H) fear memory tests. (A) There was no difference in percentage of time spent freezing between *Dll1*$^{+/-}$ (n = 11) and WT (n = 19), (B) *Lfng*$^{+/-}$ (n = 9) and WT (n = 16), (C) *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ (n = 8) and WT/WT (n = 11), (D) *Jag1*$^{+/-}$ (n = 13) and WT (n = 36) mice in the context memory test. (E–H) All mouse lines showed similar freezing levels in tone fear memory test compared to WT mice, both before (pre-CS) and during (CS) the tone. Data represent mean ± SEM.

we assessed the total distance travelled. We observed no difference in overall distance travelled in each line (HET versus WT), except in *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ mice (Fig. 4B–E, Table S3). *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ mice traveled further than their WT/WT littermate controls [$F(1,16) = 9.2$, $p < 0.05$] (Fig. 4D). Importantly, the locomotor activity of *Jag1*$^{+/-}$ mice (the only line of mice to show impaired spatial memory formation) was comparable to their WT littermates ($p > 0.05$, Fig. 4E).

Second, as anxious rodents tend to avoid exposed spaces (Prut & Belzung, 2003), we also examined the relative time mice spent in inner versus outer zones of an open field. Overall, most lines of mice showed no evidence of an anxiety-like phenotype, spending the majority of time in the outer zone of the open field [similar to their WT littermates] (Fig. 4F–I, Table S3). The one exception was *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ mice, which spent less time in outer zone than their WT littermates (Fig. 4H). An ANOVA conducted on time spent in each zone [zone 1, outer; zone 2, intermediate; zone 3, central] (see Fig. 4A), for this line yielded a significant *Genotype* by *Zone* interaction [$F(2,32) = 11$, $p < 0.001$] and a significant effect of *Zone* [$F(2,32) = 308.7$, $p < 0.001$], but no significant main effect of *Genotype* [$F(1,16) = 2.6$, $p > 0.05$]. Newman–Keuls post hoc analysis conducted on the significant interaction revealed that *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ mice spent significantly less time in the outer zone (zone 1, $p < 0.001$) and significantly more time in the intermediate zone (zone 2, $p < 0.05$), but similar time in the central zone (zone 3, $p < 0.05$) compared to their WT/WT littermates (Fig. 4H). Together, these findings suggest that *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ mice may be less "anxious" than their WT/WT littermates (hyperactive, spending less time in the outer zone of the open field). On the other hand,

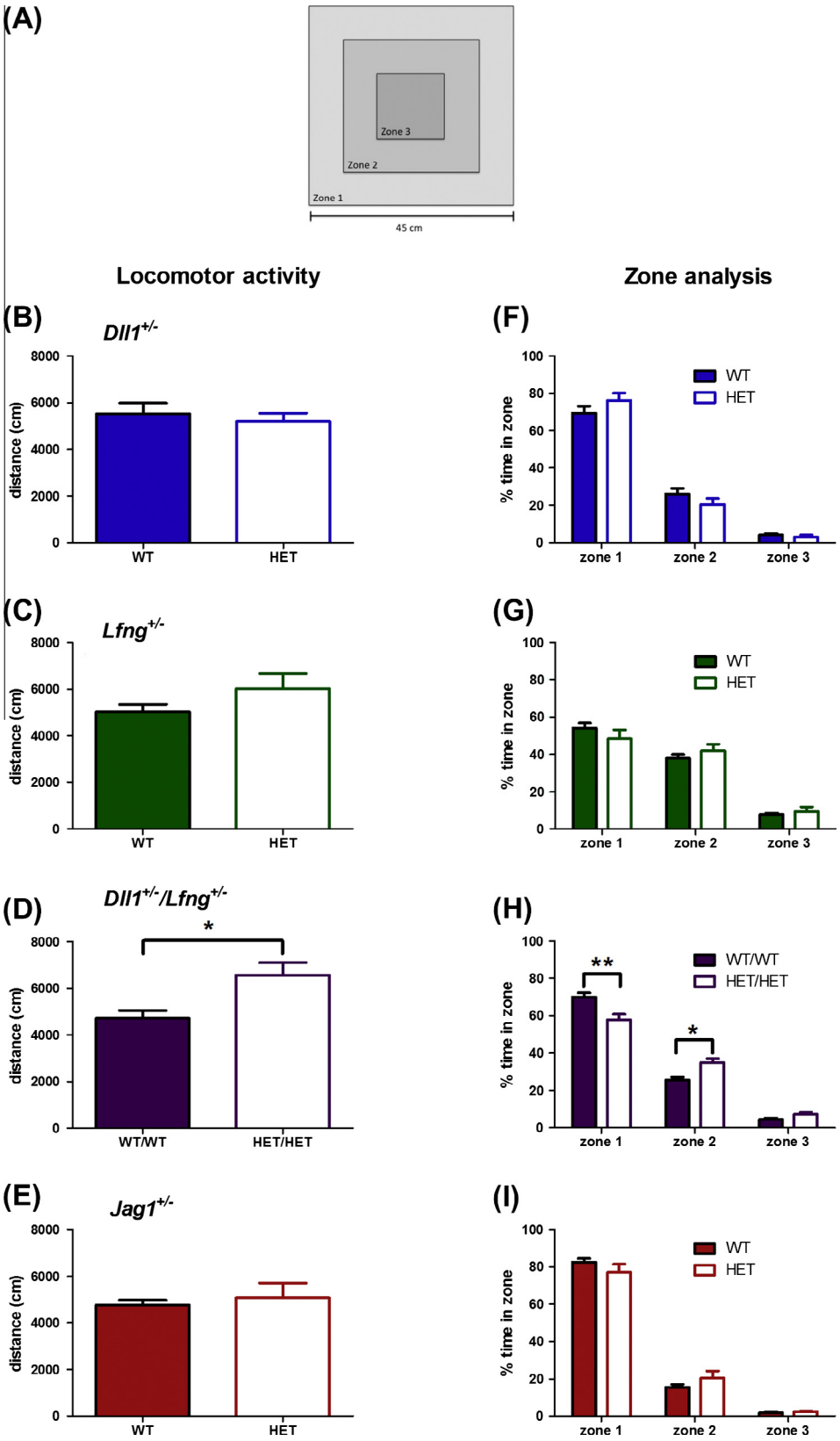

Fig. 4. Open field behavior is normal in all mice except $Dll1^{+/-}/Lfng^{+/-}$. (A) Schematic representation of the open field. (B–E) Total distance traveled by each group of mice in the open field. There was no difference in total distance traveled by, (B) $Dll1^{+/-}$ ($n = 14$) and WT ($n = 23$), (C) $Lfng^{+/-}$ ($n = 9$) and WT ($n = 16$), (E) $Jag1^{+/-}$ ($n = 16$) and WT mice ($n = 39$). (D) $Dll1^{+/-}/Lfng^{+/-}$ mice ($n = 8$) traveled a greater distance compared to WT/WT controls ($n = 10$). (F–I) Amount of time mice spent in each zone of the open field. (F) $Dll1^{+/-}$, (G) $Lfng^{+/-}$, and (I) $Jag1^{+/-}$ mice spent comparable amounts of time relative to their WT littermates in each zone of the open field. (H) $Dll1^{+/-}/Lfng^{+/-}$ mice spent less time in zone 1 (outer zone) and more time in zone 2 (intermediate zone) than their WT/WT controls, suggesting that these mice might be less anxious. Data represent mean ± SEM. $^{*}p < 0.05$. $^{**}p < 0.001$.

Jag1$^{+/-}$ mice showed normal locomotor activity and anxiety-like levels in these tests (Fig. 4E and I), indicating that the impairment in spatial memory formation observed in these mice cannot be attributed to non-specific motor effects or an increase in anxiety-like behavior.

3.4. Sensorimotor gating

Cognitive/memory impairments in many psychiatric conditions may stem from an inability to filter distracting, trivial or non-salient stimuli (Braff et al., 2001). One way to assess this process of sensorimotor gating is via prepulse inhibition (PPI) of the acoustic startle reflex. Startle responses are evoked in every mammalian species studied (including humans and mice) by loud, unexpected noises. Furthermore, the startle reflexes exhibited by humans and mice share a number of parametric characteristics (Hoffman & Ison, 1980; Ornitz, Guthrie, Kaplan, Lane, & Norman, 1986). To tap into the process of filtering irrelevant stimuli (sensorimotor gating), we assessed prepulse inhibition of the startle response in each line of mice. Prepulse inhibition measures the ability to inhibit startle response when the startle-eliciting stimulus is preceded by a non-startle eliciting prepulse stimulus (Braff & Geyer, 1990; Graham, 1975; Hoffman & Ison, 1980; Ison & Hammond, 1971).

3.4.1. Habituation of the acoustic startle response

Habituation is a form of non-associative learning in which the response to a stimulus is decreased over successive presentations (Koch, 1999). To examine habituation of the acoustic startle response in our lines of mice, we presented them with 80 trials of the same startle-inducing stimulus (120 dB white noise burst).

Over trials, all lines of mice showed decreased startle responding (habituation). To analyze these data, we divided the trials into 4 blocks (trials 1–20, 21–40, 41–60, 61–80) and conducted an ANOVA with between-subjects factor *Genotype* (WT, HET) and within-subjects factor *Trial Block* (Table S4). All lines of Notch mutant mice tested (both HET and WT littermates) showed decreased startle reactivity in response to the repeated presentations of startle stimulus over blocks, indicating normal habituation (Fig. 5A–D). Furthermore, habituation did not differ between WT and HET mice for any of the genotypes (Table S4). Therefore, habituation to a startling-stimulus was not disrupted in these Notch mutants.

3.4.2. Acoustic startle threshold

Next, we examined intensity of the acoustic stimuli required to elicit a startle response (threshold for acoustic startle response) (Koch, 1999). Mice were given acoustic stimuli of random intensities (0, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120 dB) and the average response to each intensity of startle stimulus was measured. As expected, all lines of mice showed increasing startle amplitude with increasing stimulus intensity, yielding a threshold of startle responding of roughly 95 dB (Fig. 5E–H). However, in some lines, the startle response was altered. In the *Dll1* line, HET mice showed higher levels of startle responding to some acoustic stimuli. An ANOVA revealed a significant *Genotype* by *Intensity* interaction [$F(10,240) = 1.9$, $p < 0.05$], but no significant main effect of *Genotype* [$F(1,24) = 2.1$, $p > 0.05$]. Post-hoc Newman–Keuls analysis revealed that *Dll1*$^{+/-}$ mice showed a greater startle response to stimuli of 100 and 105 dB (p's < 0.001) only over their WT littermates (Fig. 5E). In contrast, both *Lfng*$^{+/-}$ and *Lfng*$^{+/-}$/*Dll1*$^{+/-}$ mice showed a blunted startle response compared to their WT controls. For *Lfng* mice, the results of an ANOVA revealed a significant *Genotype* by *Intensity* interaction [$F(10,230) = 4.1$, $p < 0.001$], and significant main effects of *Genotype* [$F(1,23) = 4.5$, $p < 0.05$] and *Intensity* [$F(10,230) = 47.3$, $p < 0.001$]. Newman–Keuls analysis showed that *Lfng*$^{+/-}$ mice responded less to stimuli of 105 ($p < 0.05$), 110 ($p < 0.05$), 115 ($p < 0.001$) and 120 dB ($p < 0.05$) (Fig. 5F). For *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ mice, the ANOVA showed a significant *Genotype* by *Intensity* interaction [$F(10,170) = 2.3$, $p < 0.05$] but no significant effect of *Genotype* [$F(1,17) = 1.8$, $p > 0.05$]. The post hoc analysis further revealed that at intensities of 115 ($p < 0.05$) and 120 dB ($p < 0.05$), HET/HET mice showed decreased startling response compared to WT/WT mice (Fig. 5G). In contrast to these groups, *Jag1*$^{+/-}$ mice performed comparably to their WT littermates at all intensities tested ($p > 0.05$, Fig. 5H). Therefore, some lines of Notch mutants showed disruptions in the startle response.

3.4.3. Prepulse inhibition of the acoustic startle response

To measure PPI, we presented mice with a startle stimulus of 120 dB (pulse) preceded 100 ms by a low intensity (non-startle eliciting) stimulus (prepulse, 70, 75 and 80 dB). We compared the startle response of each mouse on startle alone versus prepulse trials, thus negating any possible effect of the mutation on startle alone. In all lines tested, startle reactivity decreased with increasing prepulse intensity, suggesting that all mice were able to gate their response (Fig. 5I–L). That is, PPI increased with prepulse intensity in all lines tested. An ANOVA conducted on the Percent PPI of each line of mice [between-subjects factor *Genotype* (WT, HET), within-subjects factor *Prepulse Intensity* [70, 75, 80 dB] (Table S4)], revealed that only the *Dll1/Lfng* line showed a difference depending on genotype. That is, PPI was higher in *Dll1*$^{+/-}$/*Lfng*$^{+/-}$ mice compared to their WT/WT littermates, across all pre-pulse intensities (significant effect of *Genotype* [$F(1,17) = 5.2$, $p < 0.05$] and *Intensity* [$F(2,34) = 47.5$, $p < 0.001$], but no significant *Genotype* by *Intensity* interaction [$F(2,34) = 1.9$, $p > 0.05$]). Therefore, all HET mice except *Dll1*$^{+/-}$/*Lfng*$^{+/-}$, showed comparable levels of PPI to their WT littermates.

4. Discussion

Components of the Notch pathway are widely expressed during embryonic development and, in many cases, their absence has lethal consequences. Although Notch signaling persists in the adult brain, its precise role is unknown. A number of studies have implicated the Notch pathway in learning and memory in the adult brain. For instance, the types of neuronal activity associated with memory formation and increased expression of the activity-dependent gene *Arc* (a gene important for memory formation), also engage the Notch signaling pathway (Alberi et al., 2011). Interestingly, this activity-dependent increase in neuronal Notch activation requires *Arc* (Alberi et al., 2011). Furthermore, the Notch1 receptor is crucial for learning and memory in adults (Costa et al., 2003; Ge et al., 2004; Presente et al., 2004). However, little is known about how different ligands and/or Fringe proteins contribute to memory formation. We used different lines of mice heterozygous for loss-of-function mutations in two principal Notch1 ligands (Dll1, Jag1) and Lfng, to examine specific roles for each of these Notch components in adult memory formation. Since homozygous null mutants of *Dll1* and *Jag1* are embryonically lethal (Hrabe de Angelis et al., 1997; Xue et al., 1999) and those of *Lfng* have severe skeletal malformations (Evrard, Lun, Aulehla, Gan, & Johnson, 1998; Zhang & Gridley, 1998), we took advantage of mice heterozygous for each gene. The heterozygous mutant mice developed normally and survived until adulthood. Here, we show that decreased Jag1 expression is associated with spatial memory impairment. Relative to WT mice, *Jag1*$^{+/-}$ mice showed spatial memory deficits following both strong and weak training in the water maze. This deficit cannot be attributed to non-specific deficits given that motor activity, anxiety-like behavior, and sensorimotor gating were all normal in these mice. Moreover, hippocampal-dependent non-spatial memory formation (contextual fear memory) was intact in these mice. Since an independently developed Jag1 mutant mouse line has been reported to display eye

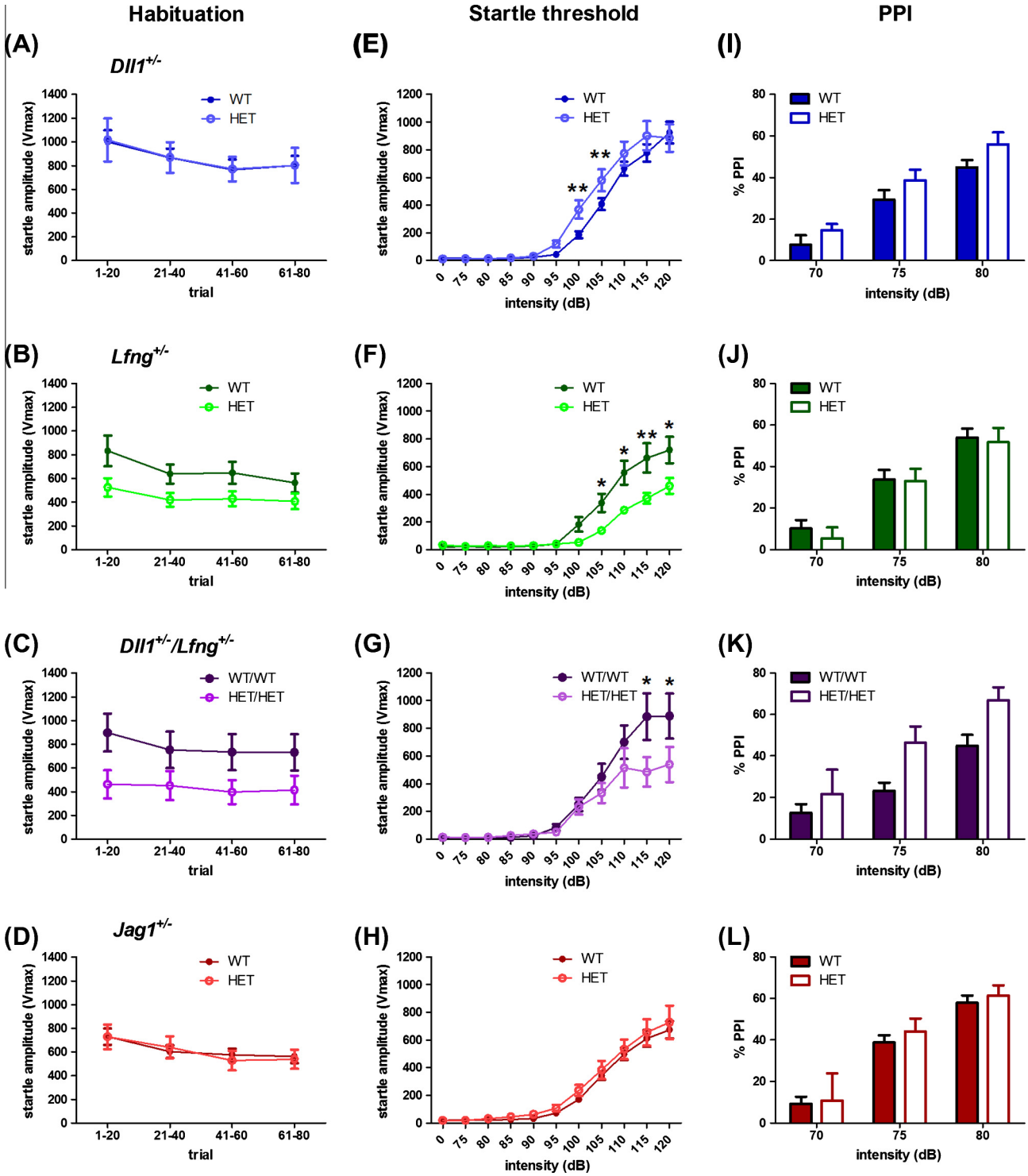

Fig. 5. Sensorimotor gating was normal in all Notch mice except $Dll1^{+/-}/Lfng^{+/-}$. (A–D) Habituation of the acoustic startle response (shown in blocks of 20 trials) are similar between (A) $Dll1^{+/-}$ ($n = 11$) and WT ($n = 15$), (B) $Lfng^{+/-}$ ($n = 9$) and WT ($n = 16$), (C) $Dll1^{+/-}/Lfng^{+/-}$ ($n = 8$) and WT/WT ($n = 11$), (D) $Jag1^{+/-}$ ($n = 16$) and WT ($n = 39$) mice. (E–H) Startle threshold levels were assessed by measuring the startle response to startle stimuli with intensities of 0–120 dB. (E) $Dll1^{+/-}$ mice showed enhanced startle reactivity above stimulus intensities of 100 and 105 dB, compared to WT mice. (F) $Lfng^{+/-}$ mice showed decreased startle reactivity at 105, 110, 115 and 120 dB stimuli compared to their WT littermates. (G) At intensities of 115 and 120 dB, $Dll1^{+/-}/Lfng^{+/-}$ mice showed lower reactivity than WT/WT mice. (H) Startle threshold levels of $Jag1^{+/-}$ mice were comparable to WT littermates. (I–L) Prepulse inhibition (%PPI) in each mouse line was assessed. PPI was tested using prepulse intensities of 70, 75 and 80 dB. No significant difference was observed in PPI between (I) $Dll1^{+/-}$ and WT, (J) $Lfng^{+/-}$ and WT, (L) $Jag1^{+/-}$ and WT mice. (K) $Dll1^{+/-}/Lfng^{+/-}$ mice showed enhanced PPI at all intensities tested compared to WT/WT mice. Data represent mean ± SEM.

dysmorphology during development (Xue et al., 1999), we carried out a visible platform version of the water maze. $Jag1^{+/-}$ mice showed a comparable performance to their WT littermates in the visible platform indicating the decreased performance in water maze could not be attributed to visual defects. A remarkably similar profile of spatial (but not contextual) memory deficits was also reported in $Notch1^{+/-}$ and $RBP\text{-}J^{+/-}$ mice (Costa et al., 2003) and mice in which Notch1 was conditionally deleted in excitatory forebrain

neurons (Alberi et al., 2011). Together, these data suggest that a chronic reduction in Jag1-Notch signaling produce specific deficits in spatial memory formation.

It is interesting to note that *Jag1* mRNA is expressed at higher levels than *Dll1* in the postnatal hippocampus (Breunig et al., 2007; Stump et al., 2002). Although these studies suggest that Jag1 may be the main Notch ligand in the adult hippocampus, consistent with its importance for spatial memory formation, a detailed analysis of Notch ligand expression throughout development and in the adult brain will be important. In our study, we cannot rule out compensatory effects of wild type alleles in heterozygous knockout mice. However, our finding that Jag1-Notch signaling is important for hippocampal-dependent spatial memory is consistent with an emerging role for Jag1 in hippocampal synaptic plasticity (Alberi et al., 2011; Wang et al., 2004). In summary, our data provide the first *in vivo* evidence that Jag1-induced Notch signaling is critical for hippocampal-dependent memory formation. Our finding agrees with several previous studies. First, application of exogenous Jag1 peptide to hippocampal slices from mice with reduced Notch levels was sufficient to rescue the hippocampal LTP deficit (Wang et al., 2004). Second, Jag1 has been localized to synapses of CA1 hippocampal neurons and is upregulated in response to neuronal activity (Alberi et al., 2011).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nlm.2013.03.001.

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