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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.