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Developmental excitatory-to-inhibitory GABA polarity switch is delayed in Ts65Dn mice, a genetic model of Down syndrome

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ABSTRACT

Down syndrome (DS) is the most frequent genetic cause of developmental abnormalities leading to intellectual disability. One notable phenomenon affecting the formation of nascent neural circuits during late developmental periods is developmental switch of GABA action from depolarizing to hyperpolarizing mode. We examined properties of this switch in DS using primary cultures and acute hippocampal slices from Ts65Dn mice, a genetic model of DS. Cultures of DIV3–DIV13 Ts65Dn and control normosomic (2 N) neurons were loaded with FURA-2 AM, and GABA action was assessed using local applications. In 2 N cultures, the number of GABA-activated cells dropped from ~100% to 20% between postnatal days 3–13 (P3–P13) reflecting the switch in GABA action polarity. In Ts65Dn cultures, the timing of this switch was delayed by 2–3 days. Next, microelectrode recordings of multi-unit activity (MUA) were performed in CA3 slices during bath application of the $GABA_A$ agonist isoguvacine. MUA frequency was increased in P8–P12 and reduced in P14–P22 slices reflecting the switch of GABA action from excitatory to inhibitory mode. The timing of this switch was delayed in Ts65Dn by approximately 2 days. Finally, frequency of giant depolarizing potentials (GDPs), a form of primordial neural activity, was significantly increased in slices from Ts65Dn pups at P12 and P14. These experimental evidences show that GABA action polarity switch is delayed in Ts65Dn model of DS, and that these changes lead to a delay in maturation of nascent neural circuits. These alterations may affect properties of neural circuits in adult animals and, therefore, represent a prospective target for pharmacotherapy of cognitive impairment in DS.

1. Introduction

Down syndrome (DS) is the most common genetic cause of intellectual disability in children and adults (Chapman and Hesketh, 2000; Dierssen, 2012; Gardiner et al., 2010; Lott, 2012; Roizen and Patterson, 2003; Wiseman et al., 2009). Cognitive impairment and other changes in DS result from the triplication of genes present on human chromosome 21 (Lejeune et al., 1959). Mouse genetic models, developed to facilitate the investigation of DS neurobiology, show multiple features characteristic of DS (Belichenko et al., 2009; Costa and Grybko, 2005; Gotti et al., 2011; Hanson et al., 2007; Jiang et al., 2015; Kleschevnikov et al., 2012c; Liu et al., 2011; Moore and Roper, 2007; Popov et al., 2011; Reeves, 2006; Salehi et al., 2007; Zhang et al., 2012).

Initial abnormalities in the brain development in DS have been noted as early as the prenatal period (Haydar and Reeves, 2012). However, the developmental profile of the brain changes suggests that the most critical alterations responsible for the intellectual disability may arise later in development, during the perinatal and/or early postnatal periods. Indeed, structural macro-parameters such as brain size and shape, as well as the activity of many enzymes and other biochemical indices, appear to be within the normal physiological range immediately after birth (Brooksbank et al., 1989; Edgin et al., 2012). Soon after birth, substantial abnormalities in the DS brain start accumulating leading to intellectual disability (Engidawork and Lubec, 2003; Golden and Hyman, 1994; Sylvester, 1983). Despite clear experimental evidence of profound early changes in DS and the importance of these changes for the development of intellectual disability, only few electrophysiological studies have examined the early developmental stages in mouse genetic models of DS (Chakrabarti et al., 2010; Mitra et al., 2012; Stern et al., 2015).

One phenomenon critically important for synaptogenesis and the early development of neural circuits is the switch of GABA action from depolarizing to hyperpolarizing mode (Ben-Ari et al., 2007). At the

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early developmental stages, GABA depolarizes neurons due to high neuronal expression of Na-K-Cl co-transporter 1 (NKCC1), which increases intracellular chloride levels above the equilibrium using the inward gradient for sodium ions (Ben-Ari et al., 2007; Watanabe and Fukuda, 2015). Subsequent activation of $GABA_A$ receptors results in the efflux of chloride ions and hence depolarization. During the second postnatal week (in rodents) GABA action switches from depolarizing to hyperpolarizing mode. This change is mainly caused by a rapid increase in the expression levels of K-Cl co-transporter 2 (KCC2), which pumps chloride ions out of neurons thus reducing intracellular chloride concentration below the equilibrium (Ben-Ari et al., 2007; Medina et al., 2014; Rivera et al., 1999; Watanabe and Fukuda, 2015). Subsequent activation of $GABA_A$ receptors results in the influx of chloride ions and hence hyperpolarization. Because depolarization favors neuronal excitation and hyperpolarization favors inhibition, this phenomenon is also frequently called "excitatory-to-inhibitory GABA switch" (Ben-Ari et al., 2007). This developmental GABA action polarity switch coincides with the most critical phases of neurogenesis and synaptogenesis, shaping the formation of neonatal neural circuits (Ben-Ari et al., 2007; Fiumelli et al., 2005; Rivera et al., 1999).

In this study, we examined properties of the developmental GABA action polarity switch in the Ts65Dn model of DS. We observed that timing of this switch is delayed in Ts65Dn *vs.* 2 N control mice. Such changes were observed in both primary cultures of hippocampal neurons and acute hippocampal slices of neonatal Ts65Dn mice. Interestingly, giant depolarizing potentials (GDPs), a type of primordial spontaneous neural activity observed in the developing brain, have been detected for a longer postnatal period in slices from Ts65Dn *vs.* 2 N littermate pups. Finally, baseline frequency of multi-unit activity (MUA) was reduced in P8-P12 Ts65Dn slices suggesting that reduced neonatal neuronal activity could be responsible, in part, for the delay of the GABA polarity switch in Ts65Dn mice. This data show that the developmental depolarizing-to-hyperpolarizing GABA polarity switch is delayed in a mouse model of DS, and that this change may affect formation of nascent neural circuits in DS.

2. Materials and methods

2.1. Animals

Segmental trisomy 16 (Ts65Dn) mice were obtained by mating female carriers of the 17^{16} chromosome (B6EiC3H-a/A-Ts65Dn) with (C57BL/6JEi X C3H/HeJ) F1 (JAX #JR1875) males (Davisson et al., 1993). Ts65Dn mice are thus maintained on the B6/C3H background. Diploid (2 N) littermate mice served as controls. For genotyping, tail samples were used to extract genomic DNA; a quantitative polymerase chain reaction protocol developed by the Jackson Laboratory, Bar Harbor, ME (http://www.jax.org) was used to measure expression of the *Mx1* gene, which is present in three copies in Ts65Dn. All mice were also screened for retinal degeneration due to *Pde6brd1* homozygosity (Bowes et al., 1993), and only animals free of retinal degeneration were used. Time-pregnant mice were housed with a 12 h light-dark cycle and *ad lib* access to food and water. The experiments were conducted in accordance with the National Institutes of Health guidelines and with an approved protocol from the University of California San Diego (UCSD) Institutional Animal Care and Use Committees.

2.2. Primary cultures of hippocampal neurons

Embryonic day 18 dissociated hippocampal neurons were obtained from Ts65Dn and 2 N timed-pregnant mice using established methods (Weissmiller et al., 2015; Zhao et al., 2014). Briefly, hippocampi were dissected in calcium and magnesium-free Hank's buffered salt solutions with 10 mM HEPES buffer and 1% Pen/Strep. Tissues were digested in trypsin (0.25% wt/vol) for 20 min at 37 °C. After 20 min of digestion, DNase I (50 μg/ml final concentration) was added for 2 min at room temperature. After three washing steps, cells were triturated through a flame-polished glass Pasteur pipette in plating medium (MEM supplemented with 5% FBS, 1× B27,1× GlutaMax, 0.6% D-(+)- Glucose). Cells were then plated (32,000 cells/cm^2) in poly-L-lysine coated 12-well dishes. After three hours, the plating medium was replaced with maintenance media (Neurobasal medium, 2% B27, 1× GlutaMax). Neurons were then maintained at 37 °C in a humidified atmosphere (95% air and 5% CO_2), and two-thirds of the medium was refreshed every other day. Cells were cultured for 3–13 days *in vitro* (DIV).

2.3. Calcium imaging

Cover slip - plated DIV3 – DIV13 neurons were loaded with the membrane-permeable fluorescent Ca^{2+} indicator Fura-2 AM (2 μM; SigmaAldrich) for 30 min at 37 °C, 5% CO_2. Following dye-loading, the cells were thoroughly washed with extracellular recording solution. Then, coverslips were placed in the recording chamber of an Olympus BX51 microscope and continuously perfused with the extracellular recording solution, pre-warmed to 37 °C and epi-illuminated at 340 and 380 nm every 2 s or, in some experiments, every 4 s. Light emitted at 510 nm was recorded, and images were collected with a QImaging Rolera – XR camera using the MetaMorph program. Pixel by pixel ratios of consecutive frames were captured, and intracellular concentration of calcium in regions of interest (ROI) corresponding to neuronal cell bodies was expressed as the ratio of emission following excitation at 340 and 380 nm (Ratio F340/F380). Analysis of sequential images was performed to follow temporal changes. After a period of basal recordings, GABA (7 s, 100 μM) and then glutamate (5 s, 100 μM) were injected in the perfusing media with an interval of 64 s. The injections were performed using a Picospritzer and two separate micropipettes, 2–3 μm tip diameter, positioned at 20–50 μm from the neurons. For analysis, averaged across the periods of the GABA and glutamate applications F340/F380 values were expressed in percentage points of the pre-application values, and the changes exceeding 1.4% were accepted as positive responses. After investigating one locus, the coverslip was moved ~350 μm to another location with fresh neurons. On each coverslip, between 50 and 150 neurons were analyzed.

2.4. Electrophysiology

Transverse hippocampal slices were prepared as previously described (Kleschevnikov et al., 2012b). Juvenile P8–P22 male pups were anesthetized with isoflurane before decapitation. The brain was quickly removed and immersed for 2 min in ice-cold artificial cerebrospinal fluid (ACSF) containing 119 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose, osmolarity 310 mOsm, continuously bubbled with carbogen (95% O_2, 5% CO_2), pH 7.4. The hippocampus was extracted and cut in ice-cold ACSF with a vibratome (Leica 1000) into 350-μm-thick slices, which recovered in oxygenated ACSF at room temperature for at least 2 h prior to experimental recordings. A slice was transferred into the recording submerged chamber and superfused with ACSF at a constant rate of 2.5 ml/min at 32 °C.

2.5. Multi-unit activity

Recordings of multiunit activity (MUA) allow for a non-invasive estimation of the spiking activity of several hundred neurons simultaneously (Cohen and Miles, 2000). To record extracellular activity, we used electrodes made of Pt/Ir wires, diameter 25 μm, imbedded in glass micropipettes made from glass capillaries (1B150F, World Precision Instruments, Sarasota, FL), with 180–200 μm-long exposed tips. To reduce the effect of the neuronal shear injury induced during slice preparation (Dzhala et al., 2012), the exposed tips of such electrodes were inserted in the middle of the CA3 pyramidal layer perpendicularly to the slice surface until the glass micropipette touched the slice surface.