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Behavioral flexibility in a mouse model of developmental cerebellar Purkinje cell loss

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ABSTRACT

Although behavioral inflexibility and Purkinje cell loss are both well established in autism, it is unknown if these phenomena are causally related. Using a mouse model, we tested the hypothesis that developmental abnormalities of the cerebellum, including Purkinje cell loss, result in behavioral inflexibility. Specifically, we made aggregation chimeras (Lc/+ ↔ +/+) between lurcher (Lc/+) mutant embryos and wildtype (+/+) control embryos. Lurcher mice lose 100% of their Purkinje cells postnatally, while chimeric mice lose varying numbers of Purkinje cells. We tested these mice on the acquisition and serial reversals of an operant conditional visual discrimination, a test of behavioral flexibility in rodents. During reversals 1 and 2, all groups of mice committed similar numbers of "perseverative" errors (those committed while session performance was ⩽40% correct). Lurchers, however, committed a significantly greater number of "learning" errors (those committed while session performance was between 41% and 85% correct) than both controls and chimeras, and most were unable to advance past reversal 3. During reversals 3 and 4, chimeras, as a group, committed more "perseverative", but not "learning" errors than controls, although a comparison of Purkinje cell number and performance in individual mice revealed that chimeras with fewer Purkinje cells made more "learning" errors and had shorter response latencies than chimeras with more Purkinje cells. These data suggest that developmental cerebellar Purkinje cell loss may affect higher level cognitive processes which have previously been shown to be mediated by the prefrontal cortex, and are commonly deficient in autism spectrum disorders.

1. Introduction

Executive function is an umbrella term for the group of closely linked, high level cognitive skills which enable the effective execution of goal-directed behaviors (Hughes, Russell, & Robbins, 1994; Pennington & Ozonoff, 1996). These skills, which include working memory, response inhibition, and behavioral flexibility, have consistently been shown to be dependent on the prefrontal cortex (PFC) in humans, non-human primates, and rodents (Dalley, Cardinal, & Robbins, 2004; Robbins & Arnsten, 2009; Robbins & Roberts, 2007). Recently, there has been considerable interest in the idea that the cerebellum, by means of its reciprocal connections to the PFC, may play a role in the performance of tasks requiring executive control. This hypothesis is supported by many functional imaging and lesion studies in humans which implicate the cerebellum in an array of executive tasks (Bellebaum & Daum, 2007; Strick, Dum, & Fiez, 2009), as well as the occurrence of cerebellar abnormalities and executive function deficits in psychiatric disorders such as autism and schizophrenia (Amaral, Schumann, & Nordahl, 2008; Andreasen & Pierson, 2008; Hill, 2004).

Behavioral flexibility, one subtype of executive function, is the ability to adapt behavior in response to changing environmental demands (Ragozzino, 2007). People with autism have considerable difficulty performing tasks requiring behavioral flexibility both in their everyday lives and on neuropsychological tests (Hill, 2004). Considering that cognitive theories of autism suggest that fundamental deficits in executive function may underlie the clinically significant symptoms of this disorder (Hill, 2004; Pennington & Ozonoff, 1996) and cerebellar neuroanatomical abnormalities are consistently found in autism (Amaral et al., 2008), determining the relationship between cerebellar abnormalities and behavioral inflexibility may provide important clues to the neural mechanisms underlying autism spectrum disorders.

We have recently reported increased activity and repetitive behavior in a mouse model designed to mimic the developmental

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cerebellar pathology observed in autism (Martin, Goldowitz, & Mittleman, 2010). In this study, we further characterize this lurcher chimera mouse model by assessing the impact of developmental cerebellar Purkinje cell loss on behavioral flexibility. Mice that are heterozygous for the lurcher spontaneous mutation (*Grid2^{Lc}*) lose virtually all of their cerebellar Purkinje cells between the 2nd and 4th weeks of development as a result of a gain-of-function mutation in the δ2 glutamate receptor gene (Caddy & Biscoe, 1979; Zuo et al., 1997). Consequently, lurcher mutants are ataxic, displaying a characteristic swaying of the hindquarters and a jerky up and down movement. Using lurcher mutant (Lc/+) and control (+/+) mice, we created chimeric mice (Lc/+ ↔ +/+) which lose a variable number of cerebellar Purkinje cells during development, depending on the incorporation of the wildtype lineage (Goldowitz, Moran, & Wetts, 1992).

The use of chimeras with a range of Purkinje cell loss conferred two important advantages over the simple comparison of Lc/+ and +/+ mice. First, in addition to comparing grouped performance of Lc/+, +/+, and chimeric mice, we were able to use correlational analysis to test for a relationship between Purkinje cell number and behavior. Second, because chimeric mice show no visible signs of ataxia unless they have lost greater than 90% of their Purkinje cells relative to controls (Martin, Escher, Goldowitz, & Mittleman, 2004), we were able to examine behavioral flexibility in mice with widely varying numbers of Purkinje cells while still controlling for the confounding effects of ataxia.

Following the creation of chimeras, we assessed performance of Lc/+ mutants, +/+ control mice, and Lc/+ ↔ +/+ chimeras on the acquisition and serial reversals of an operant conditional visual discrimination, one type of reversal learning task used to assess behavioral flexibility in rodents. Reversal learning tasks measure behavioral flexibility by assessing the ability of the subject to adapt its behavior following a reversal of stimulus-reward or stimulus–response contingencies, and have been shown to depend heavily on the PFC (Clark, Cools, & Robbins, 2004; Ragozzino, 2007).

2. Method

2.1. Subjects

A total of 25 mice were tested on the conditional visual discrimination task. This group was composed of 21 mice derived from the chimera production process and 4 mice derived from our breeding colony (2 Lc/+ mutants and 2 +/+ wildtypes). Following histological analysis, chimeric mice were assigned to genotypic groups (i.e., lurcher, chimera, or control) based upon Purkinje cell number. Lurcher mice (B6CBACa *A$^{w\text{-}J}$/A-Grid2^{Lc}*) and controls were obtained from the Jackson Laboratory (Bar Harbor, Maine) and maintained at the University of Tennessee Animal Care Facility and the University of Memphis Animal Care Facility.

2.2. Production of aggregation chimeras

Using previously described methods (Martin, Goldowitz, & Mittleman, 2003), aggregation chimeras were produced by fusing two 4–8 cell embryos derived from a mating of Lc/+ with +/+ mice of the same Lc strain background and transplanted into pseudopregnant ICR host females. All surgical procedures and animal care were in accordance with National Institutes of Health guidelines for animal welfare.

2.3. Apparatus

All training and testing was carried out in six Med Associates (www.med-associates.com) mouse operant chambers (ENV-307A). Two retractable response levers (ENV-312-2M) were mounted 19 mm above the grid floor on the left and right side of the front wall of the chamber. Two visual stimuli (ENV-221M) were located centrally on the front wall at approximately the same height as the response levers. One stimulus was positioned directly above the other, and the stimuli were positioned equidistant from the two response levers. These stimuli were modified in-house such that when illuminated, the top stimulus displayed a vertical line, and the bottom stimulus displayed a horizontal line. A house light (ENV-315M) and food receptacle which allowed access to a liquid dipper (ENV-302M) were centrally located on the rear wall. The house light was positioned 19 mm below the ceiling of the chamber, and the food receptacle was positioned at approximately the same height as the response levers and stimulus lights.

2.4. Procedure

2.4.1. General procedures

Mice were food deprived to 85–90% of baseline weight and maintained at this weight throughout the experiment by restricted feeding. Before testing, mice were acclimated to the testing chamber and trained to lever press for reward and nose poke the food receptacle at the rear of the chamber to initiate each trial. No visual or auditory stimulus explicitly signaled the mouse to initiate each trial, but mice reliably and quickly learned to do so. Mice were tested 5–6 days per week.

2.4.2. Acquisition of the conditional visual discrimination

Fig. 1 schematically illustrates the training procedure. Mice were required to nose poke the food receptacle at the rear of the chamber to begin the first trial. Following this, both levers extended into the chamber, and one of the two visual stimuli was illuminated. Levers remained extended and the stimulus light remained illuminated until a lever press was made. With the exception of correction trials (see below), the stimulus illuminated at trial onset was randomly chosen at the beginning of each trial, and was counterbalanced such that each of the two visual stimuli was presented an equal number of times during each session. If the vertical-line stimulus was illuminated, the mouse was required to press the right lever to receive a reward (0.02 mL of evaporated milk/sucrose solution). The reward was delivered by means of a liquid dipper and was available for 7 s following a correct lever press. If the horizontal-line stimulus was illuminated, the mouse was required to press the left lever to receive the reward. Training was counterbalanced such that half of the mice learned the opposite stimulus–response contingencies. Following a correct or incorrect lever press, the visual stimulus was immediately turned off and the response levers retracted. An incorrect lever press resulted in a 7 s time out during which the house light was turned off (it was on at all other times during the session) to signal that an incorrect press had occurred, and reward was omitted. A 5 s inter-trial interval with the house light on followed both reward presentation and time out, after which the mouse was required to nose poke the food receptacle to begin the next trial. Incorrect trials were followed by trials in which the same stimulus was presented repeatedly (correction trials) until the mouse made a correct choice. Sessions lasted 60 min or until the mouse obtained 72 rewards. Upon reaching the criterion of 85% correct responses during a single session, the mouse was moved to the serial reversal stage of the experiment.

2.4.3. Serial reversal learning

Each of the four reversal stages of the experiment was identical to the acquisition stage, with the exception that the stimulus–response contingencies were reversed relative to those of the previous stage. Mice had the opportunity to complete a total of four serial reversals.