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CEREBELLAR CONTRIBUTION TO HIGHER AND LOWER ORDER RULE LEARNING AND COGNITIVE FLEXIBILITY IN MICE

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Abstract—Cognitive flexibility has traditionally been considered a frontal lobe function. However, converging evidence suggests involvement of a larger brain circuit which includes the cerebellum. Reciprocal pathways connecting the cerebellum to the prefrontal cortex provide a biological substrate through which the cerebellum may modulate higher cognitive functions, and it has been observed that cognitive inflexibility and cerebellar pathology co-occur in psychiatric disorders (e.g., autism, schizophrenia, addiction). However, the degree to which the cerebellum contributes to distinct forms of cognitive flexibility and rule learning is unknown. We tested lurcher↔wildtype aggregation chimeras which lose 0–100% of cerebellar Purkinje cells during development on a touchscreen-mediated attentional set-shifting task to assess the contribution of the cerebellum to higher and lower order rule learning and cognitive flexibility. Purkinje cells, the sole output of the cerebellar cortex, ranged from 0 to 108,390 in tested mice. Reversal learning and extradimensional set-shifting were impaired in mice with ⩾ 95% Purkinje cell loss. Cognitive deficits were unrelated to motor deficits in ataxic mice. Acquisition of a simple visual discrimination and an attentional-set were unrelated to Purkinje cells. A positive relationship was observed between Purkinje cells and errors when exemplars from a novel, non-relevant dimension were introduced. Collectively, these data suggest that the cerebellum contributes to higher order cognitive flexibility, lower order cognitive flexibility, and attention to novel stimuli, but not the acquisition of higher and lower order rules. These data indicate that the cerebellar pathology observed in psychiatric disorders may underlie deficits involving cognitive flexibility and attention to novel stimuli.

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Key words: executive function, set-shifting, reversal learning, autism, chimera, cerebellum.

INTRODUCTION

Cognitive flexibility enables an organism to adapt learned behavior in the face of changing environmental demands. Deficits in this fundamental cognitive ability are proposed to underlie the maladaptive behaviors which characterize a wide range of neuropsychiatric disorders including autism (Hughes et al., 1994; Hill, 2004), schizophrenia (Pantelis et al., 1999; Floresco et al., 2009; Leeson et al., 2009), and drug addiction (Woicik et al., 2011; McCracken and Grace, 2013; Moreno-Lopez et al., 2015; Verdejo-Garcia et al., 2015; Miquel et al., 2016). Although cognitive flexibility is often discussed as a unitary construct, it can be subdivided into at least two dissociable cognitive processes. Lower order cognitive flexibility is the ability to adapt behavior following changes in lower order, stimulus-specific rules (e.g., stimulus A is correct, stimulus B is not). Conversely, higher order cognitive flexibility is the ability to adapt behavior following changes to higher order rules (e.g., stimuli from category A provide task-relevant information, stimuli from category B do not). Deficits in higher and lower order cognitive flexibility co-occur (Sahakian et al., 1990), but may also occur independently (Downes et al., 1989; Lawrence et al., 1999; Ornstein et al., 2000; Ozonoff et al., 2004). The observation that these deficits may occur independently is consistent with findings that higher and lower order cognitive flexibility are subserved, at least in part, by distinct regions of the prefrontal cortex (PFC) (Dias et al., 1996; Birrell and Brown, 2000; McAlonan and Brown, 2003; Bissonette et al., 2008).

Cognitive flexibility has traditionally been considered a frontal lobe function, although more recent evidence suggests that this view is an oversimplification. Rather, converging evidence indicates that cognitive flexibility is dependent on a larger circuit encompassing multiple brain regions including the PFC, striatum, nucleus accumbens, thalamus, and cerebellum (Ragozzino, 2007; De Bartolo et al., 2009; Floresco et al., 2009; Dickson et al., 2010; Klanker et al., 2013; Dalton et al., 2014). The Intra–Extra Dimensional Set-Shifting (IED) task, a computerized analog of the Wisconsin Card Sorting task, is commonly used to assess higher and lower order rule learning as well as the ability to adapt behavior following reversal of these rules (Sahakian and Owen, 1992). Using the IED task, the dissociable contributions

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Abbreviations: IED, Intra–Extra Dimensional Set-Shifting; mPFC, medial PFC; PFC, prefrontal cortex.

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of PFC subregions to higher and lower order cognitive flexibility have been deeply characterized in non-human primates (Dias et al., 1996, 1997; Crofts et al., 2001; Clarke et al., 2005, reviewed in Robbins and Roberts, 2007); similar findings have been reported in mice and rats using a maze-based version of the IED task (Birrell and Brown, 2000; McAlonan and Brown, 2003; Bissonette et al., 2008). However, the contribution of other brain regions to cognitive flexibility in general and cognitive flexibility subtypes specifically is only beginning to be explored. The cerebellum, in particular, has received little experimental attention in this regard, but may be a critical mediator of cognitive flexibility due to reciprocal connections with the PFC and other regions which affect higher cognitive functions (Mittleman et al., 2008; Strick et al., 2009; Watson et al., 2009, 2014; Rogers et al., 2011, 2013).

To assess the contribution of the cerebellum to higher and lower order rule learning and cognitive flexibility, we tested lurcher↔wildtype aggregation chimeras (Martin et al., 2003, 2004, 2006, 2010; Dickson et al., 2010) on a touchscreen version of the IED task that we (Dickson et al., 2014) and others (Brigman et al., 2005, 2006) have adapted for mice. Purkinje cells, the sole output of the cerebellar cortex, die during the first month of development in lurcher mutants as a result of a gain-of-function mutation in *Grid2* (Caddy and Biscoe, 1979; Zuo et al., 1997). Consequently, individual lurcher↔wildtype chimeras experience variable Purkinje cell loss ranging from 0% to 100% as a function of the incorporation of the wildtype lineage. A key advantage of this model is that the variable nature of Purkinje cell loss in individual chimeric mice enables correlational analysis of the relationship between cognitive function and cerebellar neuropathology. Moreover, precise Purkinje cell thresholds above which cognitive deficits do not occur can be identified. In the present study, lurcher↔wildtype chimeras were tested on a series of visual discriminations to assess acquisition of higher and lower order rules, as well as the ability to adapt responding following reversal of these rules. At the completion of cognitive testing, histological analysis of the cerebellum was performed and Purkinje cells were quantified. Subsequently, the relationship of Purkinje cell number and IED task performance was assessed.

EXPERIMENTAL PROCEDURES

Subjects

Lurcher mutant (B6CBACa $A^{w\text{-}J}$/A-$Grid2^{Lc}$/J) and wildtype mice were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA) and maintained at the Centre for Molecular Medicine and Therapeutics at the University of British Columbia (UBC). Aggregation chimeras were produced at UBC and shipped to the University of Memphis for behavioral testing. Mice were allowed to acclimate for at least 2 weeks prior to testing. Mice were housed in groups of 3–5 and provided free access to food until they entered the experiment at 12 weeks of age, at which point they were individually housed and food restricted to 90% of baseline weight. Mice were provided free access to water throughout the study.

Production of aggregation chimeras

Using previously described methods (Martin et al., 2003), aggregation chimeras were produced by fusing two 4-8 cell embryos derived from the mating of a lurcher mutant mouse and a wildtype mouse. All surgical procedures and animal care were performed in accordance with the National Institutes of Health guidelines for animal welfare.

IED task

Behavioral training and testing was conducted in previously described operant conditioning chambers (Dickson et al., 2013). Mice were individually housed the day before training began and were trained for at least 7 days prior to the beginning of the simple discrimination phase. Mice were tested on ten IED stages as previously described (Dickson et al., 2014). Visual stimuli used at each stage of the IED task are provided in Fig. 1. The lines dimension was relevant during the SD – IDS4R stages, and the shapes dimension was relevant during the EDS – EDSR stages. As we have done in previous

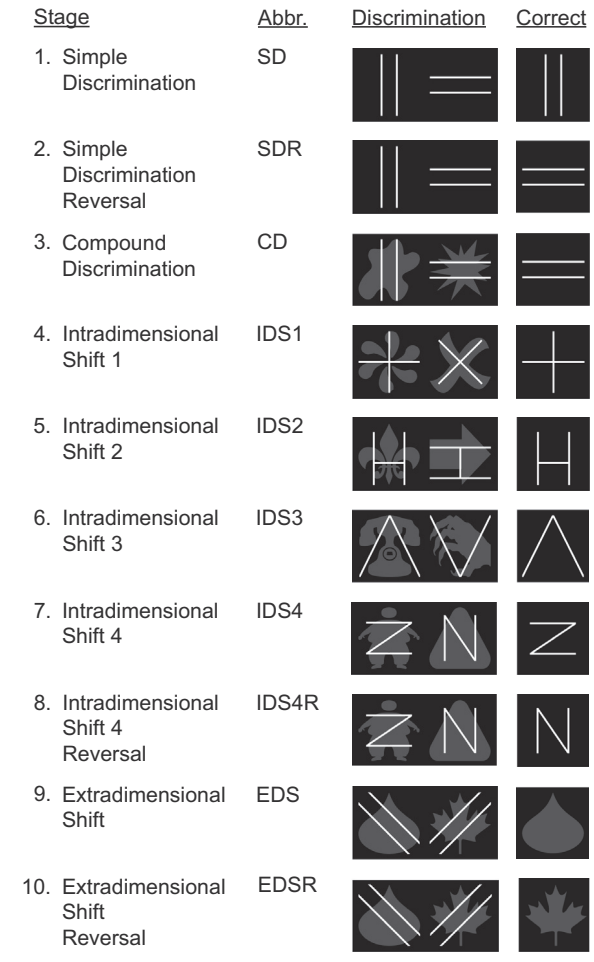

Fig. 1. Visual stimuli used at each stage of the Intra–Extra Dimensional Set-Shifting task.

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