Neuroscience 268 (2014) 212–220

AUDITORY-CUED SENSORIMOTOR TASK REVEALS DISENGAGEMENT DEFICITS IN RATS EXPOSED TO THE AUTISM-ASSOCIATED TERATOGEN VALPROIC ACID

T. CHOMIAK, * **J. HUNG, A. CIHAL,** † **J. DHALIWAL,** †
M. I. BAGHDADWALA, † **A. DZWONEK,** †
P. PODGORNY † **AND B. HU** *

Department of Clinical Neurosciences, Hotchkiss Brain Institute, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada

Abstract—Autism Spectrum Disorder (ASD) is often found to co-exist with non-core behavioral manifestations that include difficulties in disengagement of attention to sensory cues. Here we examined whether this behavioral abnormality can be induced in rats prenatally exposed to valproic acid (VPA), a well-established teratogen associated with ASD animal models. We tested rats using an auditory-cued sensorimotor task (ACST) based on the premise that ACST will be more sensitive to developmental changes in temporal association cortex (TeA) of the posterior attention system. We show that VPA rats learned the ACST markedly faster than control animals, but they exhibited a profound preoccupation with cues associated with the expectancy at the reward location such that disengagement was disrupted. Control rats on the other hand were able to disengage and utilize auditory cues for re-engagement. However, both control and VPA-treated rats performed similarly when tested on novel object recognition (NOR) and novel context mismatch (NOCM) behavioral tasks that are known to be sensitive to normal perirhinal and prefrontal network functioning respectively. Consistent with disrupted posterior rather than frontal networks, we also report that VPA can selectively act on deep-layer TeA cortical neurons by showing that VPA increased dendritic density in isolated deep-layer TeA but not frontal neurons. These results describe a useful approach to examine the role of cue-dependent control of attention systems in rodent models of autism and suggest that disengagement impairments may arise from an inability to modify behavior through the appropriate use of sensory cue associations.

Key words: autism, disengagement, temporal lobe, valproic acid, attention, savant.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by the core clinical features of impoverished communication skills, reduced disposition for social interaction, and repetitive or stereotyped behavior; and certain non-core features like difficulties in attention disengagement, sensory processing, and savantism (Kanner, 1943; Casey et al., 1993; Landry and Bryson, 2004; Baron-Cohen et al., 2009). Certain non-core and core behaviors of ASD have been ascribed to abnormal attention control. Baron-Cohen et al. (2009) have proposed that both the ''need for sameness'' and savantism that is observed in ASD may be the hallmark of strong systemizing that results from hyper-attention to detail and sensory hypersensitivity (Baron-Cohen et al., 2009). For example, being over-absorbed by ongoing activity while failing to respond to the calling of a child's own name is considered an indicator of core deficits for early ASD diagnosis (Bryson, 2005; Zwaigenbaum et al., 2005). In their study of attention behavior in children with ASD, Laundry and Bryson have suggested that deficits in attention disengagement may lead to an overly narrow beam of attention which may derive from an impairment in the posterior attention system (Landry and Bryson, 2004; Bryson, 2005).

The phenomenon of behavioral inflexibility and its relationship with sensory cueing and attention control is not well understood and there are few animal models available for the study of neural mechanisms. Sensory cues are an essential part of the cognitive system that helps us to attend, anticipate, engage, and execute particular actions (Chelazzi et al., 1993; Desimone and Duncan, 1995; Liu et al., 2000; Raz and Buhle, 2006). In humans, monkeys, and rats, the posterior attention network including the temporal association cortex (TeA) and non-lemniscal thalamocortical system plays an important role in the detection of salient sensory cues and in attention orientation through both bottom-up and top-down mechanisms (De Weerd et al., 1999; Komura et al., 2001; Corbetta and Shulman, 2002; Hu, 2003; Kimura et al., 2003; Mooney et al., 2004; Kincade et al., 2005; Chomiak et al., 2008). Distinctive sensory stimuli attract attention more effectively when they are relevant

*Corresponding authors. Address: 3280, Hospital Drive NW, Calgary, AB, Canada. Tel: +1-403-220-4486 (T. Chomiak and B. Hu). Fax: +1-403-2109550 (T. Chomiak).
E-mail addresses: tgchomia@ucalgary.ca (T. Chomiak), hub@ucalgary.ca (B. Hu).
† These authors contributed equally to this work.

Abbreviations: ACST, auditory-cued sensorimotor task; ANOVA, analysis of variance; ASD, Autism Spectrum Disorder; DAPI, 4′,6-diamidino-2-phenylindole; EDTA, ethylenediaminetetraacetic acid; HEPES, hydroxyethyl piperazineethanesulfonic acid; NOCM, novel context mismatch; NOR, novel object recognition; TeA, temporal association cortex; VPA, valproic acid.

http://dx.doi.org/10.1016/j.neuroscience.2014.02.049

to the current task, and the maintenance of focused attention is also partly dependent upon the ability to ignore irrelevant sensory distracters. This is especially important for distracter stimuli that share attributes and features or are contingent on the task which can act to disengage focused attention (Corbetta and Shulman, 2002). For instance, in monkeys, single-unit activity that reflects working memory in the posterior system, unlike that of the prefrontal system, is sensitive to intervening comparison stimuli that are relevant to the task (Miller et al., 1996). Thus, one of the key functions of the posterior attention system is to allow for the external world to modify behavior through the appropriate use of sensory cues to disengage attention (De Weerd et al., 1999; Corbetta and Shulman, 2002; Kincade et al., 2005; Posner and Fan, 2008).

Accumulating epidemiological evidence has demonstrated that *in utero* exposure to valproic acid (VPA) is associated with the emergence of an ASD phenotype (Chomiak and Hu, 2013; Chomiak et al., 2013; Christensen et al., 2013; Roullet et al., 2013). Parallel to this work, there is also a growing body of animal research literature that has used VPA as an animal model of ASD (Chomiak and Hu, 2013; Chomiak et al., 2013; Roullet et al., 2013). While the clinical data with which the prenatal VPA model is based report that women were often treated chronically with this medication during pregnancy (Moore et al., 2000; Christensen et al., 2013), the strength of examining acute exposure at a specific point during rat embryonic development is that it may help elucidate individual features and distinct behavioral manifestations associated with the disorder (Coleman and Betancur, 2005; Chomiak et al., 2013). Indeed, the acute VPA model of ASD has been widely used to study ASD-like pathophysiology in rodents (e.g. (Schneider and Przewlocki, 2005; Markram et al., 2008; Mychasiuk et al., 2012) and also see (Chomiak and Hu, 2013; Chomiak et al., 2013; Roullet et al., 2013) for review).

In this study we tested rats prenatally exposed to VPA on three behavioral tasks; an auditory-cued sensorimotor task (ACST), a novel object recognition (NOR) task, and a novel context mismatch (NOCM) task. Previous studies have shown that these tasks are differentially sensitive to TeA, perirhinal, and prefrontal cortical functioning respectively (Bussey et al., 1999; Chomiak et al., 2010; Spanswick and Dyck, 2012). We therefore hypothesized that attention disengagement would be specifically impaired in ACST in VPA-treated rats.

EXPERIMENTAL PROCEDURES

Animals

A total of 21 adult male Sprague–Dawley rats (Charles River, Wilmington, MA, USA) were used in this study and all experimental protocols were approved by the University of Calgary Conjoint Faculties Research Ethics Board. Two pregnant dams were injected i.p. with (500 mg/kg VPA dissolved in saline) and two pregnant dams were injected with an equal volume of saline for control around embryonic day 13.5, the time when the majority of deep-layer neurons are formed (Bruckner et al., 1976). Rats were cared for by the University of Calgary Animal Resource Centre and were on a 12-h light/dark cycle (7 am on 7 pm off). Standard plastic laboratory cages were used with bedding and *ad libitum* access to food and water, and were cleaned once a week. Dams were housed individually and allowed to raise their own litters until weaning on postnatal day (P) 21. Unlike control rat pups, VPA-exposed rat pups exhibited tail malformations confirming *in utero* teratogenicity of VPA (Vorhees, 1987; Favre et al., 2013).

Behavioral tests

The first behavioral task used in this study is an ACST that is sensitive to developmental changes in the TeA and is described elsewhere (Chomiak et al., 2010). Here, animals were first placed in a training cage that was divided into two locations: the cue-delivery location and the reward-delivery location on the opposite side (Fig. 1A). The rat was first allowed to familiarize itself with the new environment until it located the empty spout (i.e. no reward). The wall was covered so the animal could not see the bottle or the contents that the spout was attached to. For successful completion of a training trial, animals first had to be in the cue location. Once they were at the cue place, and prior to reward delivery, a complex sound was presented to direct the animals' attention to the reward location. Only when the animal arrived at the spout was a 30% sucrose solution was available (Fig. 1A). One rat was trained at a time and training was done every second day. Training began during the sixth postnatal week (P38) and was followed by overtraining for an additional 1.5 weeks and a 21-day retention period to ensure intact memory systems. Both groups ($n = 5$ VPA and $n = 5$ control) were then tested again and both groups demonstrated retention (not shown). Animals were water deprived for 23.5 h prior to behavioral testing but had unrestricted access to food. Following training/testing, animals had unrestricted access to water for 0.5 h. Training/testing occurred in a well-lit room with fluorescent lighting and the conditions were the same for both control and treated animals. A computer with a National Instruments A/D board (NI DAC-Card 6024E, 200 kSamples/s, 16 channels), a breakout box (National Instruments BNC-2020), and a high-speed IEEE 1394a port was used to run in-house software to trigger delivery of the complex auditory cue and latency measurements. Latency measurements represent the time difference between auditory cue presentation and arrived at the spout. A spout was always present but only when the auditory cue was presented and the animal arrived at and orientated to the spout was the 30% sucrose solution available. The latency was scored for each trial, and the average latency per trial was determined for each rat over a block of 10 trials. The same complex auditory cues were used for both groups to ensure that failure to respond to the alternate task was not stimulus-dependent.

The second series of experiments employed the NOR and the NOCM tests. Previously published work has shown that these tasks depend on the perirhinal and prefrontal cortices respectively (Bussey et al., 1999;