Neuroscience 241 (2013) 250–267

ADOLESCENT FEMALE C57BL/6 MICE WITH VULNERABILITY TO ACTIVITY-BASED ANOREXIA EXHIBIT WEAK INHIBITORY INPUT ONTO HIPPOCAMPAL CA1 PYRAMIDAL CELLS

T. G. CHOWDHURY, G. S. WABLE, N. A. SABALIAUSKAS AND C. AOKI*

Center for Neural Science, New York University, USA

Abstract—Anorexia nervosa (AN) is an eating disorder characterized by self-imposed severe starvation and often linked with excessive exercise. Activity-based anorexia (ABA) is an animal model that reproduces some of the behavioral phenotypes of AN, including the paradoxical increase in voluntary exercise following food restriction (FR). Although certain rodents have been used successfully in this animal model, C57BL/6 mice are reported to be less susceptible to ABA. We re-examined the possibility that female C57BL/6 mice might exhibit ABA vulnerability during adolescence, the developmental stage/sex among the human population with particularly high AN vulnerability. After introducing the running wheel to the cage for 3 days, ABA was induced by restricting food access to 1 h per day (ABA1, $N = 13$) or 2 h per day (ABA2, $N = 10$). All 23 exhibited increased voluntary wheel running ($p < 0.005$) and perturbed circadian rhythm within 2 days. Only one out of five survived ABA1 for 3 days, while 10 out of 10 survived ABA2 for 3 days and could subsequently restore their body weight and circadian rhythm. Exposure of recovered animals to a second ABA2 induction revealed a large range of vulnerability, even within littermates. To look for the cellular substrate of differences in vulnerability, we began by examining synaptic patterns in the hippocampus, a brain region that regulates anxiety as well as plasticity throughout life. Quantitative EM analysis revealed that CA1 pyramidal cells of animals vulnerable to the second ABA2 exhibit less GABAergic innervation on cell bodies and dendrites, relative to the animals resilient to the second ABA ($p < 0.001$) or controls ($p < 0.05$). These findings reveal that C57BL/6J adolescent females can be used to capture brain changes underlying ABA vulnerability, and that GABAergic innervation of hippocampal pyramidal neurons is one important cellular substrate to consider for understanding the progression of and resilience to AN.

Key words: anorexia nervosa, eating disorder, exercise, relapse, GABA, hippocampus.

INTRODUCTION

Anorexia nervosa (AN) is an eating disorder characterized by self-imposed severe starvation. No less than 40% and as many as 80% of the patients with AN exhibit hyperactivity in the form of excessive exercise (Epling et al., 1983; Davis et al., 1997, 1999; Hebebrand et al., 2003). This combination of symptoms leads to severe malnutrition, causing osteoporosis, amenorrhea and a mortality rate that is the highest among all mental illnesses – 10–20% (Sullivan, 1995; Birmingham et al., 2005; Bulik et al., 2007). Currently, there exists no accepted pharmacological treatment for AN, due largely to the paucity of knowledge regarding the etiology, progression and relapse of this illness.

Analysis of the AN population provides some clues regarding the etiology of AN. The incidence of AN is 10 times higher among females than males and has its first onset most commonly during adolescence. Although dieting is an almost universal phenomenon among adolescent females, only 0.5–1% of the female population are diagnosed with AN during their lifetime and the risk for AN is particularly high among those that have exhibited anxiety disorders starting in childhood (Kaye et al., 2004; Thornton et al., 2011). Results from twin studies underscore the genetic contribution to the etiology of AN (Bulik et al., 2007; Rask-Andersen et al., 2010). Moreover, this epidemiological pattern strongly suggests that there is a biological basis for the vulnerability to AN, beyond the socio-cultural factors (Kaye et al., 2009, 2011). AN is also associated with relapses that are as high as 30–50% within a year of recovery from AN (Birmingham et al., 2005), suggesting that anorexic behavior during this pivotal, final stage of brain development may cause changes in brain connections that persist beyond weight restoration.

An animal model, called activity-based anorexia (ABA), has been useful for identifying some of the vulnerability factors and changes in brain connections evoked by AN. The condition of ABA is evoked in rats by limiting food access to 1 h per day, while providing unlimited access to a running wheel. Under this condition, rats become hyperactive, voluntarily running even during the limited period of food access. This leads to a rapid weight loss and eventual death,

*Corresponding author. Tel: +1-212-998-3929; fax: +1-212-995-4011.
E-mail address: ca3@nyu.edu (C. Aoki).

Abbreviations: AN, anorexia nervosa; ABA, activity-based anorexia; BSA, bovine serum albumin; CON, control mice; DAB, 3,3′-diaminobenzidine-hydrochloride; FAA, food anticipatory activity; FR, food restriction; GAD, glutamic acid decarboxylase; GFP, green fluorescent protein; PBS, 0.01 M phosphate buffer/0.9% sodium chloride; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; SLM, stratum lacunosum-moleculare; WT, wildtype.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.03.020

unless the animal is removed from the ABA-inducing environment (Routtenberg and Kuznesof, 1967; Epling et al., 1983; Doerries et al., 1991; Dixon et al., 2003; Hillebrand et al., 2005a,b; Carrera et al., 2006; Makara et al., 2009; Aoki et al., 2012). ABA differs importantly from AN, in that food restriction (FR) is imposed by the experimenter. Animal models also cannot capture the psychological disturbances in body image or the individual's fear of gaining weight. Nevertheless, this animal model has generated insight into the biological changes evoked within the body and brain *following* starvation that may lead to voluntary hyperactivity, one of the traits that is strongly linked to the pathogenesis, progression and relapse of AN.

Although ABA was first shown in rats, it has been observed in other rodents, including the mouse (Siegfried et al., 2003; Gelegen et al., 2006, 2007, 2008, 2010; Kas et al., 2010; Lewis and Brett, 2010; Klenotich and Dulawa, 2012). Using the mouse model of ABA, Klenotich and Dulawa (2012) demonstrated that females exhibit greater vulnerability to ABA than males, thereby demonstrating that the mouse model captures the sex-linked difference in AN vulnerability. Another trait linked to AN that is captured by the mouse model is anxiety: the DBA/2J, A/J (Gelegen et al., 2007, 2010) and Balb/cJ (Klenotich and Dulawa, 2012) strains of mice exhibit greater susceptibility to ABA as well as anxiety traits.

The availability of a wide array of genetically modified mice, in addition to the relative ease for generating new genetic modifications, make the mouse a particularly ideal species for analyzing the cellular, molecular and pathway-specific signatures associated with the development of and vulnerability to AN. However, the background used most commonly for genetic modifications, i.e., the C57BL/6 strain, has been reported to be relatively less susceptible to ABA: when put in the ABA-inducing environment of wheel access and FR, these mice lose weight but reduce, rather than increase, their running wheel activity (Gelegen et al., 2006, 2007). Since the Gelegen studies used only adults, the possibility remained that these mice might exhibit ABA vulnerability during adolescence. Lewis and Brett (2010) used younger C57BL/6J mice but all were males and their ABA schedules evoked only modest or transient hyperactivity.

The current study sought to fill the gap in our knowledge by re-examining whether the C57BL/6J female mice might exhibit ABA vulnerability when FR is imposed closer to puberty onset, since this is the developmental stage/sex among the human population with higher AN vulnerability. The outcome of this study indicates that adolescent female C57BL/6J mice do, indeed, exhibit hyperactivity reliably following FR, but also that a second exposure to FR generates highly variable degrees of hyperactivity. This observation prompted us to conduct an ultrastructural study, testing the hypothesis that individual differences in ABA vulnerability might arise from differences in the inhibitory synaptic organization of the hippocampus.

Our reason for choosing to study the hippocampus was fourfold. First, the hippocampus has been recognized to undergo robust synaptic modifiability throughout life and especially during adolescence within the female brain (Smith and Woolley, 2004). Thus, we surmised that the hippocampus may be involved in the behavioral modification that followed the first exposure to ABA2. Second, our earlier study had shown increased expression of $GABA_A$ receptor subunits, $\alpha 4$ and δ at the plasma membrane of CA1 pyramidal cells following just 4 days of ABA (Aoki et al., 2012), thereby suggesting that the GABAergic system is highly and rapidly responsive to ABA induction. Third, excitability and plasticity within the CA1 field of the adolescent female hippocampus is strongly influenced by acute and chronic stress, which in turn, affects anxiety traits (McEwen et al., 1993; Shen et al., 2007, 2010). Pyramidal cells of hippocampus have also been shown to undergo morphological changes following long durations of voluntary exercise (Stranahan et al., 2009), although the response of hippocampal inhibitory neurons to exercise remains unexplored. Fourth, an animal's anxiety traits can be dampened strongly by infusing GABA receptor agonists into the hippocampus, and infusion of inverse agonists of the GABA-benzodiazepine receptors are anxiogenic (Huttunen and Myers, 1986; Kataoka et al., 1991; Talaenko, 1993). These findings point to the strong role played by GABAergic axons in the hippocampus for the regulation of anxiety. Therefore, it was reasoned that stress caused by FR and excessive voluntary activity might also prompt changes in the GABAergic input to pyramidal cells in the hippocampal CA1.

EXPERIMENTAL PROCEDURES

Animals

All procedures relating to the use of animals were according to the NIH Guide for the Care and Use of Laboratory Animals and also approved by the Institutional Animal Care and Use Committee of New York University (A3317-01).

All animals used in the study were bred at New York University's animal facility. Breeding pairs were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and consistently on a C57BL6/J background but crossed with BALB/c or Swiss Webster strains: CB6-Tg(Gad1-EGFP)G42Zjh/J (G42), which express green fluorescent protein (GFP) in parvalbumin-containing interneurons (Chattopadhyaya et al., 2004); FVB-Tg(GadGFP)45704Swn/J (GIN), which expresses GFP in somatostatin-containing interneurons (Chattopadhyaya et al., 2004), crossed at least seven generations with CB6F1/J wild-type (WT) mice at NYU; and C57BL/6J WT.

The G42 mice have been used in 19 or more studies, indicating that parvalbumin-neurons of these animals exhibit normal dendritic branching and synaptic development (Brennaman and Maness, 2008; Guan and Maness, 2010), normal experience- and activity-dependent maturation (Chattopadhyaya et al., 2004), normal excitatory/inhibitory balance (Wallace et al., 2012), normal cognitive function and network activity (Iguchi et al., 2011; Verret et al., 2012), and normal short- and long-term synaptic plasticity (Wallace et al., 2012).