Gamma Aminobutyric Acidergic and Neuronal Structural Markers in the Nucleus Accumbens Core Underlie Trait-like Impulsive Behavior

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Background: Pathological forms of impulsivity are manifest in a number of psychiatric disorders listed in DSM-5, including attention-deficit/hyperactivity disorder and substance use disorder. However, the molecular and cellular substrates of impulsivity are poorly understood. Here, we investigated a specific form of motor impulsivity in rats, namely premature responding, on a five-choice serial reaction time task.

Methods: We used in vivo voxel-based magnetic resonance imaging and ex vivo Western blot analyses to investigate putative structural, neuronal, and glial protein markers in low-impulsive (LI) and high-impulsive rats. We also investigated whether messenger RNA interference targeting glutamate decarboxylase 65/67 ($GAD_{65/67}$) gene expression in the nucleus accumbens core (NAcbC) is sufficient to increase impulsivity in LI rats.

Results: We identified structural and molecular abnormalities in the NAcbC associated with motor impulsivity in rats. We report a reduction in gray matter density in the left NAcbC of high-impulsive rats, with corresponding reductions in this region of glutamate decarboxylase ($GAD_{65/67}$) and markers of dendritic spines and microtubules. We further demonstrate that the experimental reduction of de novo of $GAD_{65/67}$ expression bilaterally in the NAcbC is sufficient to increase impulsivity in LI rats.

Conclusions: These results reveal a novel mechanism of impulsivity in rats involving gamma aminobutyric acidergic and structural abnormalities in the NAcbC with potential relevance to the etiology and treatment of attention-deficit/hyperactivity disorder and related disorders.

Key Words: Attention-deficit/hyperactivity disorder, GABA, impulsivity, magnetic resonance imaging, nucleus accumbens, psychostimulants

The concept of impulsivity encompasses a wide variety of behaviors spanning a failure of motor inhibition to individual predisposition to choose small, immediate rewards as opposed to large but delayed rewards (1,2). Deconstruction of this behavior reveals two main subgroups: 1) motor impulsivity, including motor response inhibition assessed by failure to stop an already executed response and the high occurrence of premature or anticipatory responses; and 2) decisional impulsivity, which includes delay discounting and reflection impulsivity, involving cognitive choice mechanisms and the tendency to make rapid decisions without adequate consideration of alternatives (1). High levels of impulsivity are reported in attention-deficit/hyperactivity disorder (ADHD), conduct disorder, antisocial behavior, and substance use disorder (3). Here, we focus on a specific form of motor impulsivity in rats, assessed by the number of anticipatory responses made before the onset of a visual target stimulus on a five-choice serial reaction time task (5-CSRTT) (1), a task recently validated in humans to assess impulsivity in substance addictions and binge-eating disorder (4).

The underlying mechanisms of impulsivity are not well understood but putatively involve deficiencies in norepinephrine and dopamine (DA) transmission (5–8), together with functional abnormalities in the prefrontal cortex (PFC) and striatum (9–15). Research has implicated the nucleus accumbens (NAcb) as a key brain region involved in the expression of impulsive behavior (1,16), a function postulated to involve glutamatergic inputs from the amygdala, hippocampus, midline thalamus, and PFC, together with DA inputs from the mesolimbic DA system (17) that impinge on its core (NAcbC) and shell (NAcbS) subterritories (1,16). Synaptic integration in the NAcb is governed by convergent glutamatergic and dopaminergic afferents on medium-sized, densely spiny gamma aminobutyric acid (GABA)-ergic neurons to determine behavioral output (18–20). Medium-spiny neurons (MSNs) thus play a critical role in the integration and gating of synaptic transmission in the NAcb. Surprisingly, however, few studies have investigated their involvement in the expression of impulsive behavior.

High impulsivity on the 5-CSRTT is present in 8% to 14% of the Lister-hooded rat strain and persists throughout adulthood (21,22). High-impulsive (HI) rats show escalation of intravenous cocaine and nicotine self-administration (21,23), an increased propensity for relapse after abstinence, and compulsive drug taking (24,25) compared with low-impulsive (LI) rats. High impulsivity on the

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5-CSRTT is associated with reduced availability of DA $D_{2/3}$ receptors in the ventral striatum (including the NAcb) but not the dorsal striatum (21,26). In the present study, we extend these findings using in vivo magnetic resonance imaging (MRI) and ex vivo protein analysis to isolate structural and molecular biomarkers associated with high impulsivity in rats. We report that high impulsivity on the 5-CSRTT is associated with putative alterations in dendritic spine density and is selectively and causally determined by GABA-dependent mechanisms in the NAcbC.

Methods and Materials

Subjects

We screened a total of 240 Lister-hooded rats (Charles River, Kent, United Kingdom) for low and high impulsivity on the 5-CSRTT. We selected for the present study $n = 6$ HI rats, $n = 43$ LI rats, and $n = 6$ mid-impulsive (MI) rats. The larger number of LI rats reflects their use in the glutamate decarboxylase 65/67 ($GAD_{65/67}$) antisense experiment described below. Surplus HI and MI rats were used for other studies. Subjects weighed 250 g to 275 g at the start of behavioral training and were housed in groups of four in humidity- and temperature-controlled holding rooms (22°C) under a reversed light/dark cycle (white lights off/red lights on from 7:30 AM to 7:30 PM). Rats were mildly food restricted to no more than 85% of their free feeding weights and water was available ad libitum. Experimental procedures complied with the United Kingdom Animals (Scientific Procedures) Act of 1986 and local institutional ethical guidelines.

Impulsivity Assessment

Details of the behavioral apparatus and training are provided in Supplement 1 and published elsewhere (27). Rats were trained on the 5-CSRTT to detect the location of brief visual stimuli (.7 sec) presented in a pseudorandom manner in one of five apertures. Correct responses were rewarded with a food pellet delivered in the magazine. Incorrect responses and omissions were signaled by the house light being extinguished for 5 seconds and no food delivery. A premature response was recorded if subjects responded before the onset of the stimulus and resulted in the same time-out period and loss of food reward as incorrect responses. Once rats had acquired the 5-CSRTT, they were ranked for impulsivity during a 3-week screening period. Each week consisted of 5 consecutive days of testing with days 1, 2, 4, and 5 comprising sessions each of 100 discrete trials with an intertrial interval (ITI) of 5 seconds (short ITI). During day 3, the ITI was increased to 7 seconds to increase the frequency of premature responses (long ITI). High-impulsive animals were defined as those making more than 50% of trials prematurely during each of three long ITI sessions. The lowest ranked animals were deemed LI, while rats with intermediate levels of impulsivity were deemed MI.

Morphological Assessment by MRI

Magnetic resonance imaging scanning was carried out in HI, MI and LI rats (each group $n = 6$). Rats were anesthetized with 5% isoflurane and scanned in vivo using a 4.7T Bruker BioSpec 47/40 system (repetition time/effective echo time 3500/36 msec, echo train length 8, number of excitations 2, 256 × 256 × 96 field of view, 40 × 40 × 15 mm^3, isotropic resolution 156 μm^3). A 72-mm birdcage resonator was used for transmission and signals were detected with a 20 mm diameter surface coil (Supplement 1, Morphological Assessment by MRI).

Data Processing

Our protocol for voxel-based morphometry was based on published methodology (28). Images were corrected for intensity nonhomogeneity due to the surface coil and then segmented into tissue maps corresponding to canonical gray matter (GM), white matter, and cerebrospinal fluid using SPM5 (29) (Wellcome Department of Clinical Neurology, London, United Kingdom; http://www.fil.ion.ucl.ac.uk) with the SPMMouse plugin (30). The resulting images were smoothed with an 800 μm isotropic Gaussian kernel using statistical parametric mapping and used as tissue probability maps in the unified segmentation algorithm (31).

Smoothed GM maps were fitted to a block design model to reveal differences between the LI, MI, and HI rats. A two-tailed Student *t* test was used to detect voxels where the mean GM signal differed between groups. The false discovery rate was controlled at a threshold positive false discovery rate $<.05$ as a control against multiple comparisons (32). The correlation between the GM score and impulsivity scores was determined by Pearson product-moment correlation coefficient (*r*). Williams test was used to evaluate the differences between the two dependent rho values (i.e., elements deriving from the same correlation matrix) calculated separately for the left and right hemispheres.

Western Blot Analysis

One week after the completion of MRI scanning, HI and LI rats were sacrificed by carbon dioxide inhalation; thereafter, their brains were removed and snap-frozen at −80°C. Samples of the NAcbC and NAcbS, frontoparietal cortex, and caudate putamen (CPu) were microdissected with a .75 mm^2 diameter punch from 1 mm sections of brain. Samples from one HI rat were lost during processing. Therefore, the final dataset for this aspect of the study contained $n = 6$ LI rats and $n = 5$ HI rats.

Immunodetection was performed using: 1) polyclonal rabbit anti-glial fibrillary acidic protein (Dako Cytomation, Glostrup, Denmark), a glial marker; 2) monoclonal mouse anti-Neuronal Nuclei (NeuN) (Millipore, Billerica, Massachusetts), a neuron-specific marker; 3) polyclonal rabbit anti-glutamate decarboxylase 65/67 (Millipore), the primary GABA synthesizing enzyme; 4) polyclonal rabbit anti-Neurabin II (Spinophilin; Sigma-Aldrich, St. Louis, Missouri), a dendritic spine marker; 5) monoclonal mouse anti-Microtubule Associated Protein 2 (MAP2) (Sigma), a marker for somatodendritic microtubule protein; and 6) monoclonal mouse anti-β-Actin (Abcam, United Kingdom), a housekeeping protein used as a loading control. Data analyses are described in Supplement 1, Western Blot Analysis.

Antisense Oligodeoxynucleotides

Fully deprotected and desalted phosphorothioate oligodeoxynucleotides (ODNs), purified by polyacrylamide gel electrophoresis, were purchased from Sigma. Oligodeoxynucleotides were phosphorothioated on the three terminal bases of both 5′ and 3′ ends to increase stability and minimize nonspecific toxicity. Oligodeoxynucleotide sequences and concentrations were derived from previous studies (33,34): glutamate decarboxylase 67 (GAD_{67}) antisense oligonucleotide (ASO), glutamate decarboxylase 65 (GAD_{65}) antisense, scrambled sequence control for GAD_{67}, and scrambled sequence control for GAD_{65}.

Intracerebral Cannulation

Rats destined for the glutamate decarboxylase antisense experiments were ranked for low impulsivity as described above ($n = 23$). General anesthesia was induced with isoflurane (5%) and maintained throughout the surgery at 1.5% to 2% (flow rate,