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Research Report

High novelty-seeking predicts aggression and gene expression differences within defined serotonergic cell groups

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ABSTRACT

Aggression frequently coincides with specific dimensions of emotionality, such as impulsivity, risk-taking, and drug abuse. Serotonergic (5-HTergic) neurotransmission contributes to the regulation of numerous neurobiological functions, and is thought to play a key role in modulating aggressive responses. The current study uses selectively-bred High (bHR) and Low (bLR) Responder rats that exhibit differences in emotionality and behavioral control, with bHRs exhibiting heightened novelty-induced exploration, impulsivity, and increased sensitivity to drugs of abuse, and with bLRs characterized by exaggerated depressive- and anxiety-like behaviors. Based on this behavioral profile we hypothesized that bHR rats exhibit increased aggression along with changes in testosterone and corticosterone secretion characteristic of aggression, and that these changes are accompanied by alterations in the expression of key genes that regulate 5-HTergic neurotransmission (*Tph2* and *Sert*) as well as in the activation of 5-HTergic cell groups following aggressive encounter. Our data demonstrate that when compared to bLR rats, bHRs express increased baseline *Tph2* and *Sert* in select brainstem nuclei, and when tested on the resident–intruder test they exhibited: 1) increased aggressive behavior; 2) potentiated corticosterone and testosterone secretion; and 3) diminished intrusion-induced *c-fos* expression in select 5-HTergic brainstem cell groups. The most prominent gene expression differences occurred in the B9 cell group, pontomesencephalic reticular formation, median raphe, and the gigantocellular nucleus pars α. These data are consistent with the notion that altered 5-HT neurotransmission contributes to bHRs' heightened aggression. Furthermore, they indicate that a specific subset of brainstem 5-HTergic cell groups contributes to the regulation of intrusion-elicited behavioral responses.

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1. Introduction

Serotonergic (5-HTergic) neurotransmission contributes to the regulation of virtually every neurobiological function, including sensory, motor, homeostatic, cognitive, and higher order executive functions. Furthermore, dysregulated 5-HTergic neurotransmission contributes to diverse neuropsychiatric illnesses, including major depression, bipolar disorder, suicide, sleep disorders, conduct disorders, Tourette's syndrome, and psychosis (Arango et al., 2002; Asberg et al., 1976; de Boer and Koolhaas, 2005; Lopez-Figueroa et al., 2004; Parsey et al., 2006; Rodrigo-Angulo et al., 2000; Thase, 1999). Despite such widespread functional roles, 5-HTergic neurons number only in the tens of thousands in the mammalian brain, yet these cells are highly complex, sending collateralized projections to multiple targets (Jacobs and Azmitia, 1992; Steinbusch, 1984). Emerging evidence points to functional and structural heterogeneity in the organization of these cell groups (Abrams et al., 2004; Clark et al., 2006; Jones and Light, 1992; Lowry, 2002; Lowry et al., 2008; Waselus et al., 2006). For example, 5-HTergic neurons within the caudal dorsal raphe nucleus (DRC) project to limbic targets, including septum and the bed nucleus of the stria terminalis (Waselus et al., 2006), are thought to regulate mood and affect, and are potentially involved in anxiety, depression, and suicide (Lowry et al., 2008). On the other hand, 5-HTergic neurons located more rostrally and ventrally project to the basal ganglia (Waselus et al., 2006), have been implicated in motor control and cognitive regulation, and may play a role in Tourette's syndrome and obsessive compulsive disorder (Lowry et al., 2008). Together these observations suggest the existence of parallel 5-HTergic circuits that mediate specific behaviors and physiological states.

One such behavior is aggression, which has been linked with altered 5-HTergic tone in numerous clinical studies and rodent models (Davidson et al., 2000; Kruk et al., 1998; Miczek et al., 2007; Nelson and Trainor, 2007). Interestingly, while there is a well-established link between aggression and 5-HT, the exact nature of this relationship is complex. Distinct types of aggressive displays (e.g. pathological aggression versus territorial aggression) have been associated with distinct neurochemical changes (either diminished or potentiated 5-HTergic transmission (Nelson and Trainor, 2007)). Moreover, individual susceptibility to aggression is influenced by a variety of biological factors as well as behavioral traits.

We recently developed two contrasting rodent lines — High Responder (bHR) and Low Responder (bLR) rats that were selectively-bred for their propensity to explore a novel environment. Compared to their bLR counterparts, bHR rats exhibit heightened novelty exploration, enhanced impulsivity and reward drive, as well as decreased depressive- and anxiety-like behaviors (Clinton et al., 2007; Flagel et al., 2010; Perez et al., 2009; Stead et al., 2006). These behavioral responses are reminiscent of human externalizing neuropsychiatric disorders, which also include pathological aggression (Flagel et al., 2010). Thus, in the current study we hypothesized that bHR rats exhibit heightened aggressive behavior that is correlated with altered endocrine and gene expression changes within the brainstem 5-HTergic cell groups.

2. Results

2.1. Behavior on the resident–intruder test

When exposed to the intruder, bHR residents spent markedly more time engaged in aggressive behaviors compared to bLR residents ($F_{1,33}=34.83$, $p<0.0001$; Fig. 1A). In contrast, resident bLRs spent significantly more time in defensive freezing behavior ($F_{1,33}=4.18$, $p<0.05$; Fig. 1C). No difference in aggressive behavior was detected between intruder animals paired either with bHR or bLR rats (Fig. 1B); however, time spent in defensive freezing was significantly higher in intruder rats paired with bHRs ($F_{1,33}=4.69$, $p<0.05$; Fig. 1D). Overall, bLR residents spent the vast majority of their time engaged in other (non-aggressive) behaviors, which was significantly greater than their bHR counterparts ($F_{1,33}=7.67$, $p<0.01$). Details about specific aggressive and non-aggressive behaviors are shown in Table 1.

2.2. Testosterone and corticosterone levels

Increased aggressive behavior in bHR resident rats was accompanied by a significant increase in circulating testosterone levels (Fig. 2A). In contrast, bLR residents did not increase their secretion of testosterone; consequently, post-intrusion testosterone levels were more than two-fold greater in bHR vs. bLR rats ($F_{1,38}=9.03$, $p<0.01$; Fig. 2A).

Exposure to the intruder elicited increased secretion of corticosterone in both bLR and bHR rats (main effect of intrusion compared to baseline, $F_{1,44}=181.21$, $p<0.0001$). However, this response was significantly greater in bHR animals (main effect of phenotype $F_{1,44}=4.52$, $p<0.05$ and significant phenotype × intrusion interaction $F_{1,44}=4.91$, $p<0.05$; Fig. 2B).

2.3. Characterization of raphe cell groups

Examination of *Sert* autoradiograms revealed distinct clusters of 5-HTergic cell groups throughout the brainstem. Such neurochemical parcellation agreed with cytoarchitectonic differences, as was apparent when *Sert* signal was digitally projected onto adjacent cresyl violet-stained tissue sections (Supplementary Fig. 1).

Using a combination of neurochemical and cytoarchitectonic criteria, we examined tissue sections at 240 μm intervals and identified distinct 5-HTergic cell groups throughout the brainstem (Fig. 3). We observed differences in density, intensity, and distribution of *Sert* signal associated with different cell groups. These differences also corresponded with alterations in cell size, cell shape, and packing density that were apparent in cresyl violet-stained material. Tissue processed for *Tph2* immunocytochemistry revealed the same parcellation of 5-HT-ergic cell groups as revealed by *Tph2* ISH, and similar differences in cell size, shape and density as suggested by cresyl violet stain (Supplementary Fig. 2).

Caudal 5-HTergic cell groups were located within the rostral medulla and caudal pons, while rostral groups were located within rostral pons and the midbrain (Fig. 3). Intensity and area occupied by the *Sert* signal were clearly greater within the more rostral cell groups (compare panels K–R to panels A–H in