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Research report

# Long-term behavioral effects of neonatal blockade of gastrin-releasing peptide receptors in rats: Similarities to autism spectrum disorders

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## HIGHLIGHTS

- Neonatal blockade of GRP receptors caused long term behavioral deficits.
- Rats showed reduced sociability, restrictive interests, motor stereotypies and increased fear.
- These behavioral abnormalities are similar to autism.
- Neonatal GRP receptor blockade may provide insight into autism-relevant phenotypes.

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## ABSTRACT

Gastrin releasing peptide, the mammalian counterpart of the amphibian peptide, bombesin, has been increasingly implicated in regulating normal brain function as well as in the pathogenesis of psychiatric and/or neurodevelopmental disorders. We have previously shown that the neonatal blockade of the gastrin-releasing peptide receptor (GRPr) in rats produces long-lasting consequences during central nervous system development that are commonly observed in neurodevelopmental disorders such as autism spectrum disorders. The present investigation assessed in further detail, long-term behavioral effects of neonatal GRPr blockade. During postnatal days 1–10, male Wistar rat pups ($n$ = 5–10/litter) were injected (subcutaneously) with the GRPr antagonist, RC-3095 (1 mg/kg), or a vehicle (control), twice daily. Following the drug treatment regimen, several behaviors were assessed (starting on postnatal day 14) including specific social behaviors (namely, group huddling characteristics, social interaction, and social approach), restrictive/repetitive and stereotyped behaviors (y-maze, repetitive novel object contact task, observation for stereotypies) and anxiety/fear-related responses (open field, elevated plus maze and contextual fear conditioning). Rats treated neonatally with RC-3095 showed reduced sociability, restrictive interests, motor stereotypies and enhanced learned fear response compared to the controls (vehicle-treated rats). These behavioral abnormalities are consistent with those observed in autism spectrum disorders and provide further evidence that neonatal blockade of GRPr could potentially serve as a useful model to gain a better understanding of the underlying neurodevelopmental disruptions contributing to the expression of autism-relevant phenotypes.



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## 1. Introduction

The bombesin (BB) peptide family, initially investigated as a peripheral satiety signal [1–3], has increasingly been implicated in regulating normal brain function as well as in the pathogenesis of psychiatric and/or neurodevelopmental disorders [4–7]. Gastrin releasing peptide (GRP), the mammalian counterpart of the amphibian peptide, BB, acts by binding to the GRP receptor (GRPr also referred to as BB2) located on postsynaptic neuronal membranes within the central nervous system (CNS) [4,7,8]. GRPr is widely distributed throughout the CNS with high levels of expression in the hypothalamus, brainstem and forebrain [9,10]. Of particular interest, GRPr mRNA is also highly expressed in limbic regions of the brain, including the hippocampus and amygdala [9,10], which play a major role in emotion, social interaction and attention [11–13]. Within the amygdala, GRP and GRPr mRNA are particularly abundant in the lateral nucleus [8]; a region that undergoes an abnormal pattern of postnatal development in several neurodevelopmental disorders including schizophrenia, childhood anxiety and autism spectrum disorders (ASD) [14–18].

The widespread distribution of GRP and its receptor in the brain led to intense investigation of its role in biological and behavioral functions. GRP has been found to play a role in satiety, stereotypy (i.e. grooming response), emotional learning, anxiety and social interaction [1,4,7,8,19–24]. Moreover, the GRPr system interacts with key neurotransmitters (GABA, dopamine, serotonin) [8,25–27] implicated in the pathogenesis of many disease states including Parkinson's disease, schizophrenia, major depression, anxiety disorders and autism [28–32].

Considerable evidence suggests that early manipulation of endogenous neuropeptide systems can have enduring effects on the behaviors and physiological mechanisms influenced by those peptides [33]. Given that neurodevelopmental disorders are thought to stem, at least in part, from postnatal disruption of synaptic mechanisms [34], manipulation of neuropeptide receptors during early development may represent a viable strategy in the development of animal models of a particular disorder. We had demonstrated altered anxiety-related responses in adult rats following pharmacological manipulation of the GRPr during the neonatal period [35,36]. More recently, Presti-Torres et al. [37,38] showed that neonatal blockade of the GRPr from postnatal days (PND) 1 to 10 with the GRPr antagonist, RC-3095, produced profound social deficits, impaired memory retention and decreased maternal odor preference; behavioral characteristics consistent with neurodevelopmental disorders including autism. In a subsequent study, clozapine, an atypical antipsychotic commonly prescribed to ameliorate social deficits, rescued the social impairments induced by neonatal GRPr blockade [39]. Together, these findings support the view that the GRP/GRPr system might play a critical role in the developing brain, and that early blockade of GRPr may shed light on mechanisms that underlie the expression of behavioral phenotypes observed in neurodevelopmental disorders such as autism.

In the light of these observations, coupled with findings from a genetic study [40] that identified a polymorphism (X;8 translocation occurring in the first intron) on the GRPr gene in a female patient with autism, the present study was conducted to extend the findings of Presti-Torres et al. [37,38] by further characterizing the behavioral profile of neonatal GRPr blockade with a focus on ASD-related symptoms. In this regard, core symptoms observed in ASD were targeted including impaired social interactions and stereotyped and repetitive/restrictive behavior as well as associated symptoms of ASD comprising increased fear and/or anxiety.

## 2. Methods and materials

### 2.1. Subjects

Wistar rats (Charles River, Quebec, Canada) were bred in-house. Within 24 h of birth, female pups were culled and male pups from several dams were evenly distributed across the foster mothers such that each foster litter consisted of representative pups from all dams. In Experiment 1, rats from 3 litters were fostered to 2 dams who had not been the biological parents ($n = 10$ pups/dam). In Experiment 2, rats from 6 litters were fostered to 4 dams ($n = 5$–6 pups/dam), and in Experiment 3, rats from 6 litters were fostered to 4 dams ($n = 7$–8 pups/dam). Pups were housed with dams in standard bedded plastic cages at $22 \pm 1\,^{\circ}C$, on a 12 h light-dark cycle. Pups were weaned on PND 21 and pair housed with a cagemate of the same experimental condition, with free access to food and water. Rats were weighed on PND 1–11, 15, 25, 30 and 45. A separate group of 30 male Wistar rats (age matched to test rats; Charles River Quebec, Canada) served as 'novel rats' for the social approach task.

All experimental procedures were performed in accordance with the guidelines provided by the Canadian Council on Animal Care and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Procedures were approved by the Animal Care Committee of the University of Ottawa Institute of Mental Health Research (ethics protocol number: ACC-2011-006). All attempts were made to minimize distress and number of animals used in the study.

### 2.2. Drugs and injections

The GRPr antagonist, RC-3095 (Sigma–Aldrich, Oakville, On) was dissolved in saline (vehicle; 0.9% NaCl). In Experiment 1, one litter of pups was injected subcutaneously (s.c.) twice daily (08:00 and 17:00 h) with RC-3095 (1 mg/kg) and the other with vehicle, during the first 10 postnatal days of life. In Experiments 2 and 3, in an attempt to control for potential litter effects, we distributed the treatments evenly across 4 separate litters; specifically, half of the pups ($n = 2$–3; Experiment 2 and $n = 3$–4; Experiment 3) from each of four litters were injected with RC-3095 and the other half with vehicle as described earlier (for details see Fig. 1). Pups from different treatment conditions were marked with a different pigment of food coloring on the nape of the neck. Injections were delivered at a volume of 1 ml/kg through a 30-gauge needle connected to an infusion pump. The dose of RC-3095 used was based on published studies [37,39,41]. While it is uncertain how this antagonist, when injected systemically, enters the brain to influence behavior, it has repeatedly been shown that brain function of adult rats is altered following peripheral GRPr antagonist administration [41,42]. It might be expected that such effects would be particularly prominent when antagonists are administered during the early developmental stage when the blood brain barrier is not fully developed [43].

### 2.3. Behavioral testing

Behavioral testing was conducted under low illumination (30–40 lx) between 09:00 and 13:00 h daily following a 1 h habituation to the test room, and monitored remotely via a video camera. In Experiment 1, vehicle ($n = 10$) and RC-3095 ($n = 10$) treated rats were tested in all behavioral paradigms beginning on PND 14 (with less invasive tests used first; see Fig. 1 for details). In Experiment 2, vehicle ($n = 11$) and RC-3095 ($n = 10$) treated rats were tested in three paradigms (social interaction,Y-maze and observation for stereotypies) (see Fig. 1 for details). In Experiment 3, vehicle ($n = 15$) and RC-3095 ($n = 15$) treated rats were tested in 3