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# Prefrontal cortical neuregulin-ErbB modulation of inhibitory control in rats

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ABSTRACT

Impulse control disturbances are key features of various neuropsychiatric and neurological disorders, such as attention-deficit/hyperactivity disorder, drug addiction, Parkinson disease and schizophrenia. Whereas over the last years accumulating evidence has highlighted monoaminergic modulation of the processes underlying impulse control, investigating novel mechanisms beyond monoamines may provide new intervention strategies to ameliorate impulse control disturbances. Recent work has associated the neuregulin (Nrg)-ErbB pathway with several neuropsychiatric diseases, as well as indicated its involvement in murine measures of impulse control. The aim of the present study was to investigate whether this Nrg-ErbB signaling pathway also modulates impulsive action in rats. To this end, a group of rats was trained in the 5-choice serial reaction time task (5-CSRTT), an operant paradigm that provides measures of visuospatial attention and inhibitory control processes. Upon stable baseline performance, the ErbB tyrosine kinase receptor inhibitor JNJ-28871063 (JNJ) was intracranially infused into the medioprefrontal cortex prior to test sessions. Results showed that JNJ dose-dependently improved measures of impulsive action. Importantly, other measures in the 5-CSRTT reflecting visuospatial attention or aspects of motivational behavior were not altered by JNJ. In conclusion, the present data strengthen a role for the Nrg-ErbB4 pathway in the prefrontal cortex in cognitive functioning, and in particular point towards involvement in the processes underlying impulse control.



## 1. Introduction

ErbB receptors belong to a family of receptor tyrosine kinases that are activated by ligands of the neuregulin family. Neuregulin-ErbB signaling plays an important role in neural development, including neural circuit assembly, synaptic plasticity and neurotransmission (Mei and Nave, 2014). With regard to the receptor ErbB kinase family, at least four different ErbB receptor kinases have been identified, namely ErbB1, ErbB2, ErbB3 and ErbB4 (Birchmeier, 2009). In recent years, particularly the neuregulin-ErbB4 signaling pathway has been associated with neuropsychiatric disorders, such as attention-deficit/hyperactivity disorder (ADHD), bipolar disorder, schizophrenia and nicotine dependence in genome wide association studies (Sonuga-Barke et al., 2008; Pan et al., 2011; Mei and Nave, 2014; Turner et al., 2014). Since maladaptive impulsivity and more specifically inhibitory control deficits, are shared symptoms in these disorders (Moeller et al., 2001; Gilmour et al., 2013; Ethridge et al., 2014), alterations in neuregulin-ErbB signaling may provide a novel common underlying mechanism. Indeed, in support we recently identified a gene within the neuregulin family, neuregulin 3 (*Nrg3*) that was causally related to impulsive behavior in mice (Loos et al., 2014).

To date, much of our understanding of the neural mechanisms of inhibitory control subserving impulsivity originates from rodent studies. The collective work in these models has strongly implicated various neurotransmitter systems including the monoaminergic, glutamatergic, opioid and more recently GABAergic system in inhibitory control (Pattij and Vanderschuren, 2008; Dalley et al., 2011; Caprioli et al., 2014). In addition, the medioprefrontal cortex (mPFC) and connected ventral striatal brain regions comprise a crucial neuroanatomical circuit, since lesions, reversible inactivation and functional disconnections of mPFC subregions and connected striatum impair inhibitory control

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capacities (Chudasama et al., 2003; Christakou et al., 2004; Feja and Koch, 2014).

Of abovementioned brain regions the mPFC contains high densities of ErbB4 receptor kinase, which are mainly expressed in GABA-ergic interneurons and not pyramidal neurons and are conserved in various species ranging from rodents to humans (Fazzari et al., 2010; Neddens et al., 2011). As such, ErbB4 activation has been found to promote GABA release and thereby to modulate functioning of glutamatergic pyramidal neurons and thus N-methyl-D-Aspartate (NMDA) receptor signaling (Woo et al., 2007; Chen et al., 2010; Vullhorst et al., 2015). Several studies have demonstrated glutamatergic modulation of inhibitory control in the mPFC (Mirjana et al., 2004; Murphy et al., 2005; Counotte et al., 2011; Murphy et al., 2012). Hence, this places the ErbB4 receptor kinase in the position to impact on cognitive functions, including inhibitory control.

The aim of the present study was to investigate whether, in addition to our previous murine observations (Loos et al., 2014), neuregulin-ErbB signaling in the mPFC could also contribute to inhibitory control across species in rats. For this purpose, rats were trained in the 5-choice serial reaction time task (5-CSRTT), a translational paradigm measuring aspects of visuospatial attention and inhibitory control (Bari et al., 2008). Upon stable baseline performance, intra-mPFC infusions with the ErbB kinase inhibitor JNJ-28871063 (Emanuel et al., 2008) were performed to assess its effects on visuospatial attention and inhibitory control.

## 2. Materials and methods

### 2.1. Subjects

Sixteen male Wistar rats were obtained from Harlan CPB (Horst, The Netherlands). At the start of the experiments animals weighed approximately 250 g, and were housed two per cage in macrolon cages ($42.5 \times 26.6 \times 18.5$ cm; length × width × height) under a reversed 12 h light/dark cycle (lights on at 7.00 P.M.) at controlled room temperature ($21 \pm 2$ °C) and relative humidity of $60 \pm 15$%. Animals were maintained at approximately 90% of their free-feeding weight, starting one week prior to the beginning of the experiments by restricting the amount of standard rodent food chow. Water was available ad libitum throughout the entire experiment. All experiments were conducted with the approval of the animal ethical committee of the VU University Medical Center and VU University Amsterdam, the Netherlands, and all efforts were made to minimize animal suffering and reduce the number of animals used.

### 2.2. Apparatus

Experiments were conducted in identical rat five hole nose poke operant chambers with stainless steel grid floors (MED-NPW-5L, Med Associates Inc., St. Albans, VT, USA) housed in sound-insulating and ventilated cubicles. Set in the curved wall of each box was an array of five holes. Each nose poke unit was equipped with an infrared detector and a yellow light emitting diode stimulus light. Rodent food pellets (45 mg, Formula P, Bio-Serv, Frenchtown, USA) could be delivered at the opposite wall via a dispenser. In addition, a white house light could illuminate the chamber. A computer equipped with MED-PC version 1.17 (Med Associates Inc.) controlled experimental sessions and recorded data. Animals were tested once daily from Monday until Friday, during the dark phase of the light/dark cycle.

### 2.3. Behavioral procedure 5-choice serial reaction time task

A detailed description of the 5-CSRTT behavioral procedure in our laboratory has been provided previously (Van Gaalen et al., 2006; Wiskerke et al., 2012). In short, rats were trained to detect and respond to a brief visual stimulus in one of 5 nose poke units in order to obtain a food reward. Each session terminated after 100 trials or 30 min, whichever occurred first. Initially the duration of this stimulus was 32 s and was gradually decreased to 1 s over sessions until animals reached stable baseline performance (accuracy $>80$% correct choice and $<20$% errors of omission). Responding during stimulus presentation or within the limited hold (LH) period of 2 s was counted as a correct response. Incorrect, premature responses during the fixed 5-s intertrial interval, and errors of omission (no responses or a response after the LH) did not lead to the delivery of a food reward and resulted in a 5-s time-out period during which the houselight was extinguished. Perseverative responses after correct choice, i.e., repeated responding during stimulus presentation into any stimulus unit following correct stimulus detection and before pellet collection, were measured but did not have any programmed consequences. The primary measure visuospatial attention was accurate choice calculated as [number correct trials/(correct+incorrect trials)]*100 and for inhibitory control the primary measure was the number of premature responses. In addition, the following other parameters were measured that reflect behavioral control: omission errors, i.e., the total number of omitted trials during a session; the number of perseverative responses after correct choice, measuring aspects of compulsive behavior (Robbins, 2002); response latency to make a correct choice, i.e., the mean time between stimulus onset and nose poke in the illuminated unit; and feeder latency, i.e., the latency to collect a pellet following correct choice.

### 2.4. Surgical procedure

Upon stable baseline performance in the 5-CSRTT animals were prepared for cannulation surgery by terminating the food restriction and providing free access to food for three days prior to surgery. Placement of indwelling double guide cannulae (model C235G/1.5, Plastics One, Roanoke, VA, USA) occurred under inhalation anaesthesia using a combination of oxygen (0.8 l/min) and isoflurane (1.75–2.5%; Pharmachemie BV, Haarlem, the Netherlands) in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA). Guide cannulae were positioned 1 mm above border of the prelimbic and infralimbic cortex and anchored to the skull with four stainless steel screws and dental acrylic cement. This infusion site was chosen, because our previous murine work on neuregulin-ErbB mechanisms of inhibitory control did not preferentially target a specific mPFC subregion (Loos et al., 2014). The coordinates (in mm, relative to bregma) used for placement of intracranial cannulae were A/P+3.2 mm, M/L ± 0.75 mm, D/V-3.6 mm ventral to the skull, calculated from Paxinos and Watson (1998). Rats received 0.5 ml/kg of the analgesic Ketofen (1%; Merial, Amstelveen, the Netherlands) and 0.33 ml/kg of the antibiotic Baytril (2.5%; Bayer, Mijdrecht, the Netherlands) prior to surgery. Following surgery, the animals were housed individually and had ad libitum access to food for a week before retraining in the 5-choice serial reaction time task.

### 2.5. Intracranial infusion procedure

Intracranial infusions were carried out when stable baseline performance was re-established, which took 17 sessions. Initially, during two sham infusion sessions, animals were habituated to insertion of the injectors into the guide cannulae (injectors: 33