



**Karolinska
Institutet**

Institutionen för Neurovetenskap

On the Role of Forebrain Cholinergic Innervation for Phencyclidine-induced Behaviors and Gene Expression Patterns

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska
Institutet offentligen försvaras i Petréns-salen, Nobels väg 12B.

Tisdagen den 4 juni, 2013, kl. 9.00

av

Sophia Savage

Bachelor of Arts

Huvudhandledare:

Professor Lars Olson
Karolinska Institutet
Institutionen för Neurovetenskap

Bihandledare:

Jan Kehr, PhD
Karolinska Institutet
Institutionen för Fysiologi och Farmakologi

Fakultetsopponent:

Professor Jørgen Scheel-Krüger
Aarhus Universitet
Center of Functionally Integrative
Neuroscience

Betygsnämnd:

Docent Catharina Lavebratt
Karolinska Institutet
Institutionen för Molekylär Medicin och
Kirurgi, Centrum för Molekylär Medicin

Professor Brun Ulfhake
Karolinska Institutet
Institutionen för Neurovetenskap

Professor Trevor Archer
Göteborgs Universitet
Psykologiska Institutionen

Stockholm 2013

ABSTRACT

The basalo-cortical cholinergic innervation reaches neural targets in cortex cerebri where it modulates the incoming sensory information, regulating arousal, attention, vigilance, memory and cognition. Progressive degeneration of the cholinergic neurons in the basal forebrain is considered a hallmark of Alzheimer's disease related to cognitive impairment in this condition. Dysregulation of cholinergic neurotransmission has also been implicated in schizophrenia, a chronic and debilitating neurodevelopmental disorder characterized by positive and negative symptoms and cognitive impairments, with deficits in thought processes, perceptions and emotional responsiveness. Modeling specific disruption of cholinergic function allows studies of its behavioral and molecular consequences and may contribute to the development of future therapies for Alzheimer's disease and Schizophrenia.

To investigate the role of the cholinergic system, we carried out uni- and bilateral cholinergic denervation of cortex cerebri in male Lister hooded rats using the selective immunotoxin 192 IgG-saporin to specifically target the cholinergic neurons of nucleus basalis of magnocellularis (NBM). IgG-saporin effectively removed cholinergic afferents to the cortical mantle. Intact and denervated rats were challenged with phencyclidine (PCP), a non-competitive NMDA receptor antagonist, which is known to produce schizophrenia-like psychosis in humans and hence used to model aspects of schizophrenia in rats. Experiments were conducted to determine the effects on behavior and to shed light on the underlying molecular mechanisms.

Negative symptoms of schizophrenia include social withdrawal, which is studied in animal models using social interaction tests. We found that cortical cholinergic denervation lead to a significant reduction in the duration of active social interaction in pairs of lesioned animals versus pairs of sham-operated controls. After an acute dose low of PCP (1 mg/kg, s.c.), there was a marked decrease in active social interaction for both groups, such that there was no longer a difference between lesioned and control animals. To evaluate cognitive impairments, particularly those reflecting declarative memory, a novel object recognition test was used. Neither cholinergic denervation alone, nor an acute PCP dose (1 mg/kg, s.c.) alone, blocked the ability of rats to recognize a novel object. However, animals lacking cortical cholinergic innervation and challenged with PCP were no longer able to recognize the novel object (Paper I).

Behavioral paradigms have typically been analyzed manually, though automated hardware and software systems have been introduced to speed up the process and eliminate subjective errors. However, the reliability and accuracy of computerized scoring needs to be verified for each specific paradigm. For novel object recognition, the program scoring reduced analysis time for the task, but needed manual corrections to avoid erroneous data points. Manual for scoring of social interaction revealed that this labor intensive approach was more nuanced and allowed to discern complex behaviors, whereas the automated scoring system registered more global interaction patterns. It is concluded that manual and computerized techniques for scoring social interaction offer complimentary information on various aspects of this complex behavioral task (Paper II).

The next studies were aimed to examine the bases of the behavioral responses to cortical cholinergic denervation and PCP challenge at the molecular level. PCP-induced neuronal activation was mapped using quantitative *in situ* hybridization of the neuronal immediate early gene c-Fos mRNA levels. Transcription of c-Fos responds to many different forms of activation, including stress and toxins. We found that two doses of PCP used (2 and 3 mg/kg, s.c.) caused a marked increase in neuronal c-Fos mRNA expression at 30 and 60 min after PCP administration, though these doses did not alter the levels of BDNF or Nogo receptor mRNA. Importantly, PCP responses were markedly dampened in cholinergically denervated regions of cortex cerebri (Paper III).

RNA-Sequencing was then employed to understand gene regulatory processes at a global level in somatosensory cortex with regard to effects of chronic denervation and/or a PCP challenge (1 and 3 mg/kg, s.c.). A first round of analysis focused on the genes most significantly altered by denervation (*Egr1*, *Dusp6*, *Ier2*, *Nr4a1*), PCP treatment (*Cyr61*, *Bcl6b*, *Apold1*, *Dusp1*) and those altered over time (*Sox9*, *Coq10b*, *Zfp189*, *Rnf39*) (Paper IV). The most common response was for mRNA levels to increase in response to PCP and for cholinergic denervation to dampen these increases.

The findings presented in this thesis support a role for the cholinergic system in assigning significance to incoming stimuli as exemplified by regulating the response of cortical neurons to a PCP challenge. The findings are also compatible with a proposed involvement of cholinergic dysfunction in schizophrenia.