Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

An environment-dependent modulation of cortical neural response by forebrain cholinergic neurons in awake rat

Mohammed Zacky Ariffin[a,1], Lai Seong Chang[a,1], Han Chow Koh[a,1], Chian-Ming Low[b,c,d], Sanjay Khanna[a,b,*]

[a]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore
[b]Neurobiology Program, Life Sciences Institute, National University of Singapore, Singapore 117597, Singapore
[c]Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore
[d]Department of Anesthesia, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore

ARTICLE INFO

Article history:
Accepted 23 March 2013
Available online 4 April 2013

Keywords:
Medial septum
192 IgG-saporin
C-Fos
Cingulate cortex
Hippocampus
Novelty

ABSTRACT

The forebrain cholinergic neurons project to cortex, including the hippocampus and the cingulate cortex (Cg). However, the relative influence of these neurons on behavior-linked neural processing in the two cortical areas remains unclear. We have now examined the effect of destruction of the cholinergic neurons with microinjection of the immunotoxin 192 IgG-saporin into the medial septum on the induction of c-Fos protein, an index of neuronal synaptic excitation, in the two forebrain areas to varied episodic experiences. Separate groups of rats were (a) re-exposed to the laboratory where they had previously undergone a surgery for intraseptal microinjection or (b) exposed to a novel environment. Re-exposure evoked a differential increase in the number of c-Fos positive neurons in dorsal CA1 compared to novelty, while a robust increase was observed in the Cg selectively in the novel environment. Both the differential and the selective increases were strongly attenuated by the cholinergic destruction with intraseptal-immunotoxin. These findings suggest that the cholinergic modulation of the neural processing in the two forebrain areas varies partly in an environment-dependent fashion affecting CA1 neural activation on repeat exposure to an environment where they had a relatively complex aversive experience while favoring Cg neural activation more during novelty.

1. Introduction

The cholinergic nuclei of the basal forebrain, including the medial septal region, are implicated in behavioral activation and, indeed, they are suggested to play a role in attention, associative learning and memory, sensory–motor integration, affect-motivation and cognition (Horita and Carino, 1988; Baxter et al., 1997; Ma et al., 2002; McNaughton and Corr, 2004; Sarter et al., 2005; Dwyer et al., 2007; Bland, 2009; Easton et al., 2011; Fuller et al., 2011; Lee et al., 2011). The medial septal region, consisting of the medial septum, the vertical and the horizontal limbs of the diagonal band of Broca, is anatomically and functionally linked to cortex, especially to the allocortex hippocampus (Kiss et al., 1990). Stimuli that

*Corresponding author at: Department of Physiology (MD9), Yong Loo Lin School of Medicine, National University of Singapore, 2 Medical Drive, Singapore 117597, Singapore. Fax: +65 6778 8161.
E-mail addresses: phsks@nus.edu.sg, sanjay_khanna@nuhs.edu.sg (S. Khanna).
[1]Contributed equally to the work performed. SK contributed to the writing of the manuscript together with C-ML and MZA.

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.03.046

affect septal neurons, including the cholinergic neurons also potently excite neurons in the hippocampus. In this regard, the septo-hippocampal cholinergic neurons are activated in rats on exposure of the animals to behaviorally arousing stimuli of varied hue including exposure to open field (Dudar et al., 1979; Imperato et al., 1991; Inglis and Fibiger, 1995; Thiel et al., 1998; Ceccarelli et al., 1999; Giovannini et al., 2001). Likewise, arousing stimuli, especially animal exposure to novel open field, elicit the expression of *c-fos* and the corresponding protein, c-Fos, in the neurons of the hippocampus, *c-fos* being an immediate early gene (IEG) that is expressed in neurons on synaptic excitation (Emmert and Herman, 1999; Pace et al., 2005; Sheth et al., 2008; VanElzakker et al., 2008). The effect is marked and consistent in the hippocampal field CA1. Additionally, both the septal cholinergic neurons and acetylcholine exert a strong modulatory influence on hippocampal neural activity (Zheng and Khanna, 2001; Leung et al., 2003).

Exposure to arousing stimulus, such as novelty also evokes an activation of other structures including the amygdala and the cingulate cortex (Cg; Nagahara and Handa, 1997; Salome et al., 2004; Hale et al., 2008). Moreover, the medial septum, including the septal cholinergic neurons and the hippocampus projects to these regions, while the amygdala and the Cg project, directly and/or indirectly, back to the hippocampus (Stewart et al., 1985; Senut et al., 1989; Kiss et al., 1990; Petrovich et al., 2001; Risold, 2004; Cenquizca and Swanson, 2007; Jones and Witter, 2007). The interaction between these regions, for example between septal cholinergic neurons and Cg, may have bearing on aspects of animal behavior that is consistent with the idea that the above-mentioned regions are interrelated components of an affective-cognitive network (Marston et al., 1994; McNaughton and Corr, 2004).

We have used c-Fos expression in the present study to examine the relative pattern of activation of the two cortical structures, namely hippocampus and the Cg, to two different environmental milieus and their modulation by the cholinergic neurons. The influence of septal cholinergic neurons was examined by destroying these with the immunotoxin, 192 IgG-saporin. The immunotoxin-induced septal lesion have a profound effect on cholinergic markers in both the hippocampus and the Cg suggesting that such lesions affect key cholinergic afferents to the two cortical regions.

2. Results

2.1. *Effect of 192 IgG saporin on ChAT positive neurons in the medial septal region*

The average number of ChAT positive neurons in the medial septal region of the representative control, namely the *IS veh nov* group, were 1538.00±156.60 neurons ($n=7$; range 919–2176; Fig. 1). On the other hand, robust destruction was observed following IgG pretreatment (e.g. Fig. 1). Thus, the total number of ChAT positive neurons in the two test groups, namely the *IS IgG Nov* and the *IS IgG reex* were 39.00±13.07 ($n=8$) and 71.00±13.33 ($n=6$), respectively. The counts in the two test groups were significantly different from the control but were not different from each other (Groups, $F_{2,18}=88.78$, $P<0.0001$, 1-way ANOVA followed by Newman–Keuls test). In all cases the IgG-induced loss was >90% when compared to the average number of ChAT positive neurons (1538.00) in the control group.

Despite the near absence of ChAT positive neurons, robust parvalbumin staining was still observed suggesting a relatively selective effect of the neurotoxin on septal cholinergic neurons (Fig. 1).

2.2. *Effect of environmental exposure on the number of c-Fos like immunoreactive (FLI) neurons in whole hippocampus and Cg*

Compared to the control groups (*IS veh basal*), the number of FLI positive neurons were increased in the whole CA1 subfield

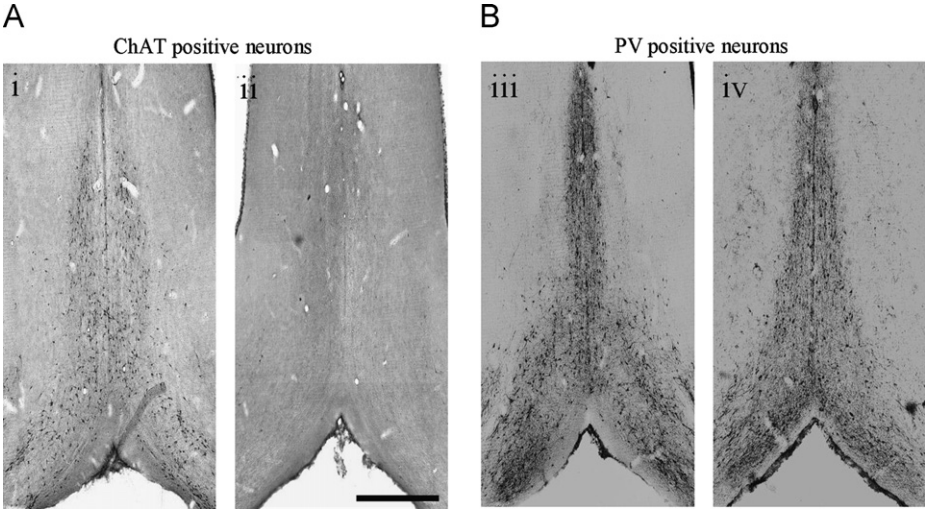

Fig. 1 – Intra-septal immunotoxin 192 IgG-saporin (0.8 μl of 0.42 μg/μl) selectively destroyed choline acetyl transferase (ChAT, A) positive cholinergic neurons while largely sparing parvalbumin (PV, B) positive GABAergic septohippocampal neurons. The panels are digitized images (600 dpi) through the medial septum of animals treated with intra-septal vehicle (Ai and Biii) or the immunotoxin (Aii and Biv). Cells stained for ChAT or PV stand out as darkly stained relative to the background. Scale bar represents 100 μm.