Behavioural Brain Research 292 (2015) 219–232

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Behavioral and cognitive changes after early postnatal lesions of the rat mediodorsal thalamus

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HIGHLIGHTS

- Early insult of the mediodorsal thalamus (MD) disturbed cognitive behaviors.
- Early MD damage decreased locomotor activity, and reduced social interactions.
- Early insult of the MD disturbed postnatal maturation of affective behavior.
- The MD is important for prefrontal cortex function during brain maturation.

ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form 8 June 2015
Accepted 9 June 2015
Available online 12 June 2015

Keywords:
Lesion
Mediodorsal thalamus
Anxiety
Learning
Prefrontal cortex
Schizophrenia

ABSTRACT

Early insults to the thalamus result in functional and/or structural abnormalities in the cerebral cortex. However, differences in behavioral and cognitive changes after early insult are not well characterized. The present study assessed whether early postnatal damage to mediodorsal nucleus of the thalamus (MD), reciprocally interconnected with the prefrontal cortex, causes behavioral and cognitive alterations in young adult rats. Rat pups at postnatal day 4 received bilateral electrolytic lesion of MD, or a MD Sham lesion or were anesthetized controls; on recovery they were returned to their mothers until weaning. Seven weeks later, all rats were tested with the following behavioral and cognitive paradigms: T-maze test, open field test, actimetry, elevated plus maze test, social interactions test and passive avoidance test. Rats with bilateral MD damage presented with disrupted recognition memory, deficits in shifting response rules, significant hypoactivity, increased anxiety-like behavior, deficits in learning associations as well as decreased locomotor activity, and reduced social interactions compared to MD Sham lesion and anesthetized Control rats. The lesion also caused significant decreases in pyramidal cell density in three frontal cortex regions: medial infralimbic cortex, dorsolateral anterior cortex, and cingulate Cg1 cortex. The present findings suggest a functional role for MD in the postnatal maturation of affective behavior. Further some of the behavioral and cognitive alterations observed in these young adult rats after early MD lesion are reminiscent of those present in major psycho-affective disorders, such as schizophrenia in humans.

1. Introduction

There is increasing interest in modeling some of the cognitive deficits in schizophrenia with paradigms that are suitable for both humans and experimental animals. These cognitive deficits include impairments in working memory and behavioral flexibility [1,2]. Altered activity in the prefrontal cortex (PFC) has been associated with these cognitive symptoms [3–5]. However, the cognitive symptoms of schizophrenia, typically linked to prefrontal cortex (PFC) dysfunction remain essentially resistant to treatment

Abbreviations: MD, mediodorsal nucleus of the thalamus; PFC, prefrontal cortex; ZT, Zeitgeber time; P4, postnatal day 4; AP, antero-posteriority; L, laterality; P, profoundness; US, unconditioned stimulus; CS, conditioned stimulus; R, Ratio; ATN, anterior thalamic nucleus; VTA, ventral tegmental area; SA, spontaneous activity.

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http://dx.doi.org/10.1016/j.bbr.2015.06.017

[6]. Thus, it remains critical to understand the underlying neural mechanisms of these symptoms in order to target effective treatments, because cognitive symptoms are highly predictive for the long-term prognosis of the disease [7,8].

Recently, the mediodorsal thalamus (MD) has become a focus of attention in the study of cognitive symptoms and schizophrenia, mainly due to its dense excitatory reciprocal connections with the PFC [9]. Neuroimaging and postmortem anatomy in schizophrenic patients indicate that MD is shrunken with neuronal loss, or has metabolic changes [10,11]. Behavioral neuroscience studies using monkeys or rodents have indicated that MD contributes to learning, memory, decision-making, fear conditioning, anxiety, and impaired social interactions [8,12–20]. Further early postnatal unilateral electrolytic lesions of MD at postnatal day 4 disrupt dendritic development of PFC pyramidal cells [21]. This evidence from animal models suggests that changes to MD observed in patients with schizophrenia may be associated with some of the cognitive symptoms associated with this neuropsychiatric disorder.

Given that schizophrenia is a developmental disorder, traditionally associated with dysfunction in the PFC, the longer-term cognitive and behavioral effects of neonatal lesions within the medial PFC have been studied in rodent models. Interestingly, rats with PFC lesions induced at postnatal day 7 have deficits in cognitive control-like behaviors [22], but intact working memory, suggesting that early developmental damage to the PFC can lead to impaired executive functioning but does not impact on memory. Thus far, after early onset damage to the MD, adult rats display reduced vertical activity in an open field test [23] but learning remained intact using an operant delayed alternation task [24]. However, further assessments of changes in their affective state and cognition have not been conducted.

Thus, the present study tested the longer-term cognitive and behavioral effects of early developmental insult to MD in adult rats. A battery of cognitive and behavioral tasks assessed the generality of social interactions and anxiety, as well as learning and memory. We hypothesized that if the influence of the MD is critical for the normal, ongoing development of the PFC from the earliest postnatal stages of brain maturation then MD surgical lesions induced just after birth should cause long-term alterations in cognition and behavior in adult rats.

2. Material and methods

2.1. Animals

The experiments involved 67 Sprague–Dawley rats, bred in the central animal care facilities of Cadi Ayyad University, Marrakech, Morocco. During the training sessions, five animals were excluded from the study, because they showed a high level of anxiety (freezing); thus, the final number was 60 rats (47 males/13 females; sex was counter balanced between groups). After birth, the rats (weighing 6 g ±2) were housed with their mothers in litters and kept under constant temperature conditions (20 °C ±2), using a 12 h light/12 h dark cycle (lights on at 7 am: ZT 0), with water and food ad libitum. Among these 60 rats, 18 rats were used for the delayed alternation tasks, actimetry, and for the quantification of the PFC cells density, while the remaining 42 rats were used for the assessments of the other behavioral tasks described below.

The study received approval of the Council Committee of research laboratories of the Faculty of Sciences, Cadi Ayyad University of Marrakech. All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63). All efforts were made to minimize any animal suffering.

2.2. Surgery

Previous brain developmental studies have documented that in rodents, MD afferents reach the PFC between postnatal days 1–7, while PFC efferents reach the MD during postnatal days 4–9 [25]. Thus the MD lesions were performed on postnatal day 4 (P4) in order to alter the communication between the MD and PFC during this critical period of postnatal brain development. Date of birth was designated as postnatal day P0. The animals were randomly divided into three groups ($n = 19$ per group): group 1, pups received a bilateral electrolytic lesion in MD; group 2, MD Sham lesion (electrode placement only); group 3, naïve (unlesioned) but anesthetized Controls. On P4, all pups were anesthetized by hypothermia for 5 min [23] and placed in an adapted stereotactic apparatus using aseptic conditions. In groups 1 and 2, a skin incision was made, just lateral to the midline (to avoid the midsagittal sinus), their scalp was then retracted, and the skull, bregma and the intended lesion site were gently exposed. The electrode was inserted through the skull, which is still very soft at day P4 using the following coordinates from bregma: AP = −1 mm, $L = \pm 0.5$, and $P = -3.5$ mm. (These coordinates had been previously determined in pilot neurosurgeries in a different cohort of P4 rats in which the electrode track placement was histologically verified using thionin blue-stained serial sections). In the animals of group 1 only, a current of 5 mA (tip negative) was then passed for 3 s. In groups 1 and 2, the electrode was removed, and then the skin was repositioned and held in place with super-glue (ARADINE, Morocco). After surgery, the wounds were cleaned by betadine 10% (Pharma Laboratory, Morocco) and the pups were warmed up and returned to their mothers, where they remained until weaning at postnatal day 30. During the post-weaning period, groups of 5 rats were housed together per cage (40 cm × 25 cm × 18 cm). Seven weeks post-surgery (P53), animals were trained in the behavioral paradigms and assessments were made for any changes to their behavior and cognition.

2.3. Histology

After the behavioral testing was completed, the animals ($n = 60$) were deeply anesthetized with urethane 40% (1 g/kg, from Sigma–Aldrich, France), and then transcardially perfused with saline (0.9%), followed by 4% paraformaldehyde in phosphate-buffered saline (0.2 M). The brains were extracted from the skull and postfixed in the fixation solution for 12h, cryoprotected overnight in 30% sucrose, and then sectioned in coronal plane at a 40 μm thickness using a cryostat (Leica Microsystems, Germany). Serial sections through the thalamus were Nissl-stained using thionin blue.

2.4. Cell density measurements

In the rat, the MD projects to medial prelimbic cortex, dorsolateral anterior cortex and cingulate Cg1 cortex. The slides containing these areas of interest were determined using cresyl violet stained sections based on gross anatomical boundaries according to Faul and Mehler [26]. We referred to Paxinos et al. [27] to locate the gross borders of medial prelimbic cortex. To distinguish the cytoarchitectural borders of dorsolateral anterior cortex and cingulate cortices we used criteria from Gabbott et al. [28]. All of the sections containing the prefrontal regions were analyzed for cell density. Cells were counted in three evenly spaced sections with a random start. Contours were traced around each region of interest and a counting grid superimposed on the contour. Every third intersection with a random start was marked for counting. A counting box of 100 × 100 μm (with a buffer zone of 5 μm corresponding to the exclusion line on either surface was employed to exclude the cut