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Research report

Cerebellar nuclei are involved in impulsive behaviour

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ABSTRACT

Recent anatomical and clinical evidence has shown that the cerebellum, primarily considered a motor control structure, is also involved in higher cognitive functions and behavioural changes, such as impulsive behaviour. Impulsive behaviour has been shown in several studies to be increased by lesions of the mediodorsal (MD) thalamic nucleus. We performed deep brain stimulation (DBS) of the mediodorsal and ventrolateral (VL) thalamic nuclei in rats, clinically mimicking such a lesion, and tested them for changes in impulsive behaviour in a choice reaction time test. We then analysed the effects of this stimulation on c-Fos expression in both the deep cerebellar nuclei (DCbN) and the prefrontal cortex (PFC), and correlated these outcomes to the measured changes in impulsive behaviour. DBS of the MD thalamic nucleus increased impulsive behaviour without changing motor parameters. This was accompanied by a decrease in the c-Fos expression in all cerebellar nuclei; with a corresponding increase in c-Fos expression in the PFC. DBS of the VL thalamic nucleus caused no significant change in behaviour or c-Fos expression in either region. The present study demonstrates that impulsive behaviour involves the cerebellar nuclei, possibly through a decreased selective attention caused by a disruption of the cerebello-thalamo-cortical pathways through the MD thalamic nucleus.

1. Introduction

Since the first description of the cerebellum as a motor structure by Flourens in the beginning of the nineteenth century, the cerebellum has been regarded to be involved in motor coordination and control [24]. Inputs from several brain regions including the motor, premotor, posterior parietal, cingulate and prefrontal cortex (PFC) were shown to converge on the cerebellar cortex and deep cerebellar nuclei (DCbN) [2,53]. It was thought that the deep cerebellar nuclei project strictly to a specific region in the ventrolateral thalamic nucleus (VL), and from there to the primary motor cortex [4,31]. The last two decades, however, there has been increasing attention for a possible non-motor role of the cerebellum. Leiner and associates first hypothesized that the disproportional increase in size of the phylogenetically newer parts of the cerebellum in anthropoid apes and humans might imply a role of the cerebellum in mental skills in the same manner as the phylogenetically older parts of the cerebellum contribute to motor skills [35]. Neuroanatomical research has shown that the cerebellum projects to the PFC through the VL and other thalamic nuclei including the mediodorsal thalamic nucleus (MD) [46,63] and the reticular nucleus of the thalamus (RNT) [14]. These anatomical connections between the cerebellum and the PFC suggest that the cerebellum is involved in non-motor circuits.

Other findings supporting the theory of a non-motor role of the cerebellum come from the clinical setting. This was first pointed out by case reports and case series describing behavioural disturbances in patients with cerebellar lesions due to for example stroke or tumours [5,25,36,51]. Nowadays there is an increasing

Abbreviations: MD, mediodorsal thalamic nucleus; DBS, deep brain stimulation; VL, ventrolateral thalamic nucleus; DCbN, deep cerebellar nuclei; PFC, prefrontal cortex; RNT, reticular nucleus of the thalamus; IN, interposite nucleus of the cerebellum; FN, fastigial nucleus of the cerebellum; DN, dentate nucleus of the cerebellum; CCAS, cerebellar cognitive affective syndrome; CMS, cerebellar mutism syndrome; OCD, obsessive compulsive disorder; HFS, high frequency stimulation; RT, reaction time; MT, motor time; PRr, premature response ratio.

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amount of information from human neuroimaging studies to support this view of a non-motor role of the cerebellum [54,60]. In this respect, Schmahmann and colleagues have described a constellation of cognitive deficits in a group of patients with selective cerebellar damage which has been termed the Cerebellar Cognitive Affective Syndrome (CCAS) [54]. This syndrome consists of impairment of executive functions, difficulties with spatial cognition, changes of personality including disinhibited or inappropriate behaviour, and language deficits. A similar syndrome has been described in children after resection of cerebellar tumours. This cerebellar mutism syndrome (CMS) is characterized by mutism in combination with other neurological, cognitive and behavioural deficits including either decreased initiation of activity or disinhibition [12,13,36,62]. It has been suggested that CCAS and CMS are in fact different presentations of the same syndrome [36,62]. Additional evidence that the cerebellum plays a role in higher-order behaviour comes from imaging studies showing significantly smaller cerebellar volumes in patients with neuropsychiatric diseases such as ADHD and schizophrenia [3,9,32,52,59], and altered metabolism in the cerebellum in patients with obsessive compulsive disorder (OCD) [11,30,47,50]. There is also an abnormal cerebellar activation on functional MRI in autistic subjects during motor and cognitive tasks [1]. Pathological findings in these subjects include loss of Purkinje cells (PCs) [48]. Impulsivity is a core feature of these neuropsychiatric diseases [33]. As described above, there have been several reports of changes in impulsive behaviour as part of the CCAS or CMS in patients with cerebellar damage [5,36,54]. In autism spectrum disorders the degree of explorative behaviour is correlated to the volume of vermal lobules VI–VII of the cerebellum [49]. Duchenne's muscular dystrophy has been shown to have a heightened association with ADHD, autism spectrum disorders and OCD [28], which is speculated to be caused by the absence of dystrophin in the cerebellum [18]. Furthermore, King and co-workers showed a change of activity in the cerebellar vermis in a rat model of ADHD after treatment with a 5-HT agonist that is known to relieve impulsivity [33].

These recent findings suggest a key role of the cerebellum in impulsive behaviour. In this study we have tested this hypothesis. Previous research has shown that lesions of the MD induce an increase in impulsivity in rats as measured by premature responding in a choice reaction time test [16]. High frequency stimulation (HFS) has been shown to cause a net inhibition of the target region, an effect that is clinically comparable to a lesion [8], although the underlying mechanisms are probably different. The benefit of HFS above lesions is that it is reversible and adjustable. Therefore, we chose to perform electrical stimulation of the MD thalamic nucleus in rats as a behavioural model of impulsivity, in which premature responses serve as a measure of inhibitory control [57]. To evaluate the specificity of the site of stimulation, we stimulated the VL thalamic nucleus. DBS of the VL thalamic nucleus is known to effectively reduce various types of tremor in patients [41]. Case series show a subtle memory effect of VL thalamic DBS [39,61], but to the author's knowledge changes of impulsivity were never reported. Finally, activation in the DCbN and the PFC was evaluated using c-Fos immunohistochemistry. We focussed on the cerebellar nuclei as these are the output structures of the cerebellum. c-Fos was chosen as it is an immediate early gene which is considered to reflect neuronal activation [55,56]. The expression of c-Fos is maximal about two hours after administration of a stimulus, and disappears again after four to eight hours even if the stimulus is continued [55]. Therefore the c-Fos expression found two hours after a specific stimulus can be considered to be the direct effect of this stimulus, and this expression can be used to identify brain areas directly influenced by this stimulus [56].

2. Material and methods

2.1. Animals

We used male Lewis rats (12 weeks old, bred and housed at the Central Animal Facility of the Maastricht University, The Netherlands), with an average body weight of 300 g. The rats were housed individually in standard cages with sawdust bedding in an air-conditioned room (about 20 °C) under a 12/12-h reversed light/dark cycle. During testing (5 days a week), the rats were given 10–20 g of food (standard laboratory chow; Hopefarms, Woerden, The Netherlands) a day; the other 2 days they were left to rest and fed ad libitum. This schedule reduced their weight to approximately 90% of their free feeding weight during the week. The rats had free access to water at all times. All experiments were approved by the Animal Experiments and Ethics Committee of the Maastricht University.

2.2. Surgical procedure

Rats were randomly assigned to one of the following groups: control (no surgery; $n = 8$), bilateral VL electrode implantation ($n = 10$), or bilateral MD electrode implantation ($n = 10$). A detailed description of the surgical procedure has been reported previously [58]. In brief, animals were anaesthetized throughout the entire procedure using a combination of ketamine (90 mg/kg) and xylazine (10 mg/kg) injected subcutaneously. Rats were placed in a stereotactic apparatus (Stoelting, Wood Dale, USA; model 51653). Two burr holes were made in the skull immediately above the targets to allow for insertion of electrodes in the MD thalamic nucleus (coordinates from bregma: AP = −2.8 mm, ML = ± 0.6 mm, $V = -5.0$ mm) or in the VL thalamic nucleus (coordinates from bregma: AP = −2.8 mm, ML = ± 1.5 mm, $V = -6.5$ mm). A construction of two stainless steel electrodes (Technomed, The Netherlands), both concentric and bipolar, with a tip diameter of 50 μm and a shaft diameter of 250 μm, was implanted in this experiment. The electrodes were fixed in position using dental cement (Heraeus Kulzer, Germany).

2.3. Deep brain stimulation

The stimulus was delivered using a World Precision Instrument (WPI, Berlin, Germany) accupulser (A310) and a stimulus isolator (A360). Real time verification of the parameters applied during stimulation was obtained for both electrodes of the bilateral construction, using a digital oscilloscope (Agilent Technologies, Agilent 54622D oscilloscope, The Netherlands). Stimulation started a few minutes before the test and lasted for the duration of each session. The rats had 12–72 h without stimulation between sessions.

2.4. Behavioural evaluation

A choice reaction time task was performed as described previously [57]. In summary, rats were tested in Skinner chambers (inner dimensions: 40 cm × 30 cm × 33 cm) equipped with two retractable levers with cue lights just above the levers. A food tray (5 cm × 5 cm and 2.5 cm above the grid floor) was positioned equidistant between the two levers and could be accessed by pushing a hinged panel. The levers (4 cm wide) projected 2 cm into the conditioning chamber and were located 6 cm from both sides of the food tray and 12 cm above the grid floor. A light and a loudspeaker were fixed in the ceiling of the conditioning chamber. The test procedure was controlled by a personal computer and the data stored on disk at the end of a session (time precision = 1 ms). During a trial, the rat had to insert its nose into the central panel until a tone sounds. A high tone (10 kHz; 80 dB) required the rat to press the left lever and a low tone (2.5 kHz; 80 dB) required pressing of the right lever. The period (randomly chosen from 0.6 to 1.5 s, with steps of 0.1 s) between nose insertion and tone was termed the hold duration. When a rat did not hold the panel for the entire hold duration, the same interval was repeated when the panel was next pushed. Depression of the lever resulted in food reward in only 50% of cases to increase the motivation of the animals for the task [10]. The reinforcement was given independently of the reaction time. After pushing the panel there was a lapse of ten seconds before the next trial could be started. Each session lasted 30 min or until the completion of 60 trials. The following parameters were analysed:

Reaction time (RT) is defined as the latency between the onset of the tone and the release of the tray panel. It is generally accepted that RTs shorter than 100 ms are unlikely to be true reaction times, and RTs longer than 1500 ms should not be considered to be task-related; therefore these RTs were discarded [10].

Motor time (MT) is defined as the latency between the release of the tray panel and the lever press. It was assumed that MTs longer than 2 s did not reflect 'true' motor time; these were left out of the analysis [10].

Premature responses ratio (PRr) is defined as the total number of times the rat released the tray panel before the hold duration had elapsed, divided by the number of trials. After a premature response the rats had to start the same trial again by pushing the hinged panel.

Prior to surgery, the rats underwent a period of two weeks of preliminary training until the mean reaction time of performances was stabilized, after which the baseline behavioural performance was evaluated [10].