Electrophysiological Characterization of The Cerebellum in the Arterially Perfused Hindbrain and Upper Body of The Rat

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Abstract In the present study, a non-pulsatile arterially perfused hindbrain and upper body rat preparation is described which is an extension of the brainstem preparation reported by Potts et al., (Brain Res Bull 53(1):59–67), 1. The modified in situ preparation allows study of cerebellar function whilst preserving the integrity of many of its interconnections with the brainstem, upper spinal cord and the peripheral nervous system of the head and forelimbs. Evoked mossy fibre, climbing fibre and parallel fibre field potentials and EMG activity elicited in forelimb biceps muscle by interpositus stimulation provided evidence that both cerebellar inputs and outputs remain operational in this preparation. Similarly, the spontaneous and evoked single unit activity of Purkinje cells, putative Golgi cells, molecular interneurones and cerebellar nuclear neurones was similar to activity patterns reported in vivo. The advantages of the preparation include the ability to record, without the complications of anaesthesia, stable single unit activity for extended periods (3 h or more), from regions of the rat cerebellum that are difficult to access in vivo. The preparation should therefore be a useful adjunct to in vitro and in vivo studies of neural circuits underlying cerebellar contributions to movement control and motor learning.

Keywords Purkinje cell · Climbing fibre · Mossy fibre · Parallel fibre · Nucleus interpositus

Introduction

The cerebellum is an ideal brain structure to study neuronal network mechanisms as its connections are well characterised. It is the largest sensorimotor structure within the CNS and is intimately involved in the regulation of smooth and accurate movements. It also contributes to our ability to improve movements through experience and practice, and an increasing body of evidence suggests that it may also be involved in non-motor processes such as sensory, autonomic and cognitive functions (see [2, 3] for details). In view of the remarkable structural uniformity of its intrinsic circuitry, it is likely that the cerebellum performs a similar computation on the information it receives irrespective of the specific function in question [4]. Diversity of function between different cerebellar regions is thought to arise primarily from differences in regional input and output connectivity (e.g. [5]).

In terms of inputs, many in vivo electrophysiological studies in anaesthetised, decerebrate and awake animals have shown that sensory information arising from peripheral receptors in muscle, skin and joints can be highly effective at altering on-going neural activity in the cerebellum (e.g [6–9]). Related anatomical studies have also provided important details regarding the ‘wiring’ of brain circuits linking the cerebellum to other major structures within the CNS; both in terms of cerebellar inputs, notably the inferior olive [10] and the pontine nuclei, [11]; and also in terms of cerebellar outputs e.g. the red nucleus [12].
By comparison, the ability to manipulate the ionic environment and high mechanical stability of in vitro brain slice preparations and tissue-cultured neurones has provided important details of the cellular mechanisms and synaptic events that underlie cerebellar function (e.g. [13–16]). However, an important limitation of such preparations is that they usually lack neuronal connections with other parts of the CNS, and invariably deprive the cerebellum of its links with the rest of the body. Indeed, normal cerebellar function is dependent on maintaining its afferent and efferent connections with other brain structures (especially the inferior olive, e.g. [17–19]).

We have therefore assessed the utility of a non-pulsatile, perfused hindbrain and upper body preparation (PHBP) to study the neuronal mechanisms of the intact cerebellum in situ. Previously, a similar preparation (but lacking a cerebellum) has been used to examine brainstem mechanisms that underlie cardio-respiratory control [1, 20]. Other cerebellar preparations have succeeded in providing good recording stability whilst maintaining major links between the cerebellum and brainstem [21–27], but the in situ preparation described here also maintains interconnectivity with the peripheral nervous system and muscles of the upper body, thereby allowing a study of cerebellar activity in the context of intact neural systems.

Methods

Surgical Procedures

Figure 1 shows a schematic diagram of the experimental setup. All surgical and experimental procedures were approved and performed in accordance with local animal welfare

![Fig. 1. Schematic diagram of the perfused hindbrain and upper body preparation of the rat. The chamber is constructed from Perspex and held in place on a steel table with magnetic feet. The rat is decerebrated and perfused with a modified Ringer’s solution gassed with 95% O₂/5%CO₂ mixture via a double lumen cannula inserted into the descending aorta. Perfusion pressure (PP) is monitored via the second lumen of the cannula. The rat is held in place with ear and nose bars. A peristaltic pump is used to circulate the perfusate. Two drain holes in the bottom of the chamber return the perfusate efflux to the perfusate reservoir for reperfusion. Tygon tubing is used to connect the circuitry. Arrows indicate the direction of perfusate flow. The flow rate of the perfusate was altered until phrenic nerve activity (PNA) monitored via a suction electrode displayed a eupneic (ramp-like) pattern. PNA was integrated to show more clearly the rhythmic activity consistent with eupnoea. Extra- and intracellular recordings were made from individual cerebellar neurones. Electrical stimulation of the brachial plexus or cerebellar cortex was used to evoke cerebellar field potentials. Forelimb muscle contraction evoked by microstimulation of nucleus interpositus was monitored by EMG recordings from the ipsilateral biceps brachii muscle. See “Methods” for further details.](image)
guidelines and the UK Animal (Scientific Procedures) Act 1986. Ten minutes prior to the commencement of the experiment, the animal received a 0.1–0.2 ml intraperitoneal injection of heparin (1,000 mg/ml) to prevent the coagulation of blood during preparation. According to Paton's [20] original description in the mouse, 25 Wistar rats of either sex weighing between 85–120 g (4–6 weeks of age) were deeply anaesthetised with halothane and bisected below the diaphragm. The upper half of the body was immediately transferred to ice-cold modified Ringer's solution bubbled with carbogen. The rostral brain was exposed and the preparation was decerebrated rostral to the superior colliculus by aspiration or with a blunt spatula. With the exception of the forepaws and face, the preparation was skinned to prevent fur from blocking the tubing as well as pathogens contaminating the perfusate and degrading the preparation. Within the thorax, the descending aorta was dissected free, the phrenic nerve cut at the level of the diaphragm and the lungs removed. The preparation was then transferred to a custom made Perspex recording chamber held in place on a steel table by magnetic feet (Fig. 1 inset). The descending aorta was rapidly cannulated with a double lumen cannula (DLR-4, Braintree Scientific, MA, USA) for retrograde perfusion of modified Ringer's solution (125 mM NaCl, 24 mM NaHCO₃, 10 mM dextrose, 5 mM KCl, 2.5 mM CaCl₂, 1.25 mM MgSO₄, 1.25 mM KH₂PO₄, pH7.4) containing 1.25% of the oncotic agent Ficoll 70 or 1% Polyethylene glycol. All chemicals were purchased from Sigma-Aldrich. The second lumen of the cannula was used to monitor perfusion pressure via a pressure transducer. The cannula was held in place by securely tying a length of Tygon tubing to the unitating agent vecuronium bromide (0.04 μg/ml, Norcuron, Organon Teknica, Cambridge, UK), was added to the perfusate to block neuromuscular transmission. In two experiments, harmaline was added to the perfusate (5 μg/ml, Sigma-Aldrich) to assess the effects of a pharmacological challenge on the olivo-cerebellar system.

Once the perfusion pressure was stable, the animal was secured in place by atraumatic ear bars and a nose clamp attached to the Perspex bath. By removing the intraparietal and occipital bone, the entire surface of the cerebellum was exposed (including regions not normally accessible in vivo, such as the anterior lobe and the most lateral parts of the hemispheres). The dura was carefully removed. The circulating perfusate prevented the surface of the cerebellum from drying out. A 30 gauge T-type thermocouple wire (W-TW-30, Physitemp Instruments, Clifton, NJ, USA) was inserted just rostral to the cerebellum to monitor brain temperature via a microprobe thermometer (BAT-12, Physitemp Instruments, Clifton, NJ, USA) and maintained at approximately 32–34°C.

Stimulation Methods

In individual experiments one or more of the following stimulation procedures was carried out: (1) stimulation of cerebellar cortical parallel fibres was achieved with a glass-insulated tungsten microelectrode insulated up to the tip (tip diameter 15–25 μm) and positioned at or just below the surface of the cerebellar cortex and in line with the recording electrode placed at a distance 0.5–1.0 mm away in the long axis of the folium. Cathodal square pulses of 0.1 ms duration and up to 15 μA in strength were delivered every 2 s. (2) To evoke climbing fibre responses in the C1 zone of the paramedian lobule [28] bipolar percutaneous needles were inserted into the ipsilateral brachial plexus. Square wave constant-voltage electrical stimulation of 0.1 ms duration was delivered every 2 s at intensities <5 V which was sufficient to generate a small but visible twitch of the forelimb. (3) Stimulation of mossy fibres was achieved by dissecting free the facial nerve from the surrounding connective tissue and the exposed tips of Teflon-coated silver wires were wrapped around the nerve for electrical stimulation (0.2 ms constant voltage square wave pulses, <15 V, or 0.1 ms constant current square wave pulses, <2 mA delivered once every 2 s). The prepared
nerve was then embedded in fast-curing dental impression to prevent it drying out (Xantopren, Heraeus Kulzer, NY).

(4) To stimulate nucleus interpositus, a concentric bipolar electrode was used. Cathodal constant current pulses (0.2 ms, 100–200 μA) were delivered once every 2 s. At some cerebellar nuclei sites, pulse trains (0.1 s, 300 Hz) were also employed. To verify the location of the stimulating electrode in nucleus interpositus, the cerebellum was removed at the end of the experiment (typically 4–5 h after the initial decerebration) and sunk in a solution of 10% formaldehyde and 20% sucrose. Sagittal sections were cut on a freezing microtome at a thickness of 100μm. Sections were mounted serially onto slides, counterstained with cresyl violet, and dehydrated, cleared and coverslipped and scrutinised with a light microscope.

Recording Parameters and Data Processing

In every experiment the respiratory motor pattern was monitored by recording phrenic nerve activity via a glass suction electrode. Phrenic nerve signals were amplified and band-pass filtered (0.08–3 kHz). In most experiments, extracellular recordings from single cerebellar neurones were made with high impedance glass-insulated tungsten microelectrodes (3–5 MΩ). For intracellular recording glass micropipettes containing 3 M KCl or 3 MK-acetate were used (40–80 MΩ). Signals were differentially amplified and band-pass filtered (0.1–10 kHz). In additional experiments, field recordings from the cerebellar surface were made with low impedance glass-insulated tungsten microelectrodes (~50 kΩ). These signals were differentially amplified and band-pass filtered at 0.3–5 kHz. The ipsilateral forelimb response to interpositus stimulation was monitored by EMG recordings with a pair of fine needle electrodes inserted into the biceps brachii muscle. EMG activity was amplified and filtered (0.1–5 kHz) through a differential amplifier. Perfusion pressure and electrophysiological recordings were digitised at 4 and 20 kHz respectively via a data acquisition system (Micro1401 or 1401plus, Cambridge Electronic Design, UK) that was interfaced with a computer running customised Spike2 software (Cambridge Electronic Design) for off-line analysis. Single-unit spikes were discriminated using Spike2 template matching. Interspike interval (ISI) histograms and autocorrelograms were computed for each cerebellar neurone using a bin width of 1 ms and 2 ms respectively and a 180 s sampling interval. The coefficient of variation, (CV=standard deviation ISI/mean ISI) was used to describe the regularity of the resting discharges [29]. A high CV signifies a spike train with high ISI variability, whereas a low CV value indicates a more regular pattern of firing. All mean values given in “Results” are expressed as mean±standard error mean (SEM).

Results

Evoked Field Potential Responses

The threshold for evoking cortical responses by parallel fibre stimulation is well known to be highly sensitive to the viability of the cerebellum [25, 30]. We therefore used local cortical electrical stimulation to evoke parallel fibre volleys in five preparations (see “Methods” for further details). As shown in Fig. 2a, electrical stimuli applied to the surface of the cerebellar cortex elicited a brief triphasic positive-negative-positive response, characteristic of compound action potentials propagating in a bundle of parallel fibres recorded in the molecular layer [30]. Threshold currents ranged between 3–5μA. When the recording electrode was moved 300 μm off line from the stimulating electrode, the triphasic response was no longer evident, consistent with earlier reports that the volley is restricted to a narrow ‘beam’ of parallel fibres [31, 32]. Conduction velocities were determined from the peak of the initial positive wave. In the example illustrated in Fig. 2a, the conduction velocity was 0.36 ms⁻¹. For all five experiments conduction velocities were on average 0.32 ± 0.04 ms⁻¹. These values are in good agreement with those reported previously for in vivo preparations for e.g. 0.33–0.5 ms⁻¹ [30, 33] but are faster than those reported in vitro for e.g. 0.2–0.3 ms⁻¹ [34].

In three preparations, the response of individual Purkinje cells to parallel fibre stimuli was also observed and consisted of a single action potential (a simple spike) with an onset latency of 2.2 ± 0.26 ms (n=3) (Fig. 2b). Such responses could be evoked with threshold currents below 5 μA, suggesting that parallel fibre excitatory synaptic input to Purkinje cells was operating normally in the PHBP [cf. 35].

To assess the integrity of mossy fibre inputs, in three experiments we recorded responses evoked by facial nerve stimulation in the molecular and granular layers of lobule IXa [cf. 36–38]. In the granular layer, stimulation of the ipsilateral facial nerve evoked an initial diphasic positive-negative wave (P1-N1, Fig. 2c, lower panel) which relates to the arrival of the mossy fibre volley [38]. A later negative wave (N2, Fig. 2c) has been ascribed to the synaptic activation of granule cells and Golgi cells [38] while the consequent activation of the parallel fibres by the granule cells results in a P2 wave with an average latency of 4.6 ms±0.54 (n=3). As in previous studies, an additional early positive response attributed to the brainstem was evident before the P1 wave, and did not reverse polarity between the molecular and granular layers [36, 37]. By contrast, and consistent with previous studies, the P2 wave was found to reverse polarity in the molecular layer to produce a negative-going deflection (N3, Fig. 2c, upper panel).
To assess the integrity of climbing fibre inputs to the cerebellum we electrically stimulated the ipsilateral brachial plexus, and recorded evoked field potentials (Fig. 2d) in the forelimb receiving area of the cerebellar cortex, located in the C1 zone of the paramedian lobule [28]. These responses were located in the medial part of the paramedian lobule and had an onset latency of 9.4±0.6 ms (n=3). The waveform, cortical location and onset latency of these field potentials were characteristic of climbing fibre responses evoked in the cerebellar cortical C1 zone as described previously in vivo in a number of species [e.g. cat, 39, rat, 28, 40, ferret, 41]. Moreover, when pairs of responses were evoked with supramaximal stimuli delivered at intervals between 20–150 ms (the paired pulse test), the second response displayed a reduction in amplitude (Fig. 2f). This is an additional feature of climbing fibre evoked field potentials [42, 43]. Consistent with climbing fibre responses, a rebound facilitation was also present at longer interstimulus intervals (Fig. 2f). Note also that the gradable nature of the evoked responses indicates that they were not unitary but represented the summed activity of a population of cerebellar neurones (Purkinje cells). As with previous studies in vivo [36], when shorter latency (~3–5 ms) responses attributable to activation of mossy fibres were evoked by the peripheral stimulation, these were much less reduced in amplitude by the paired pulse test and were even increased in some instances (Fig. 2e).
To assess the integrity of cerebellar output in the PHBP in two preparations we used microstimulation in nucleus interpositus and recorded evoked EMG activity in the ipsilateral forelimb. Electrical stimulation of nucleus interpositus either with single pulses or with trains of stimuli could readily evoke EMG activity in the ipsilateral forelimb muscle biceps brachii (latency 10–20 ms, Fig. 3a). As the stimulating electrode was advanced into the cerebellum the stimulus intensity required to evoke a detectable EMG response progressively decreased until a site was found that elicited EMG activity with a minimum threshold (200–300µA). Systematic study of threshold levels as a function of stimulus frequency was not carried out, nevertheless, it was noted that an increase in the frequency of the pulse train resulted in a decrease in the stimulus threshold as reported previously in vivo [44, 45]. Application of a neuromuscular blocker to the perfusate abolished the EMG responses, indicating that they were generated by neuromuscular synaptic transmission. Histological reconstruction of the stimulation site indicated that

in both experiments the electrode tip where minimum stimulation currents were used was located in nucleus interpositus anterior (Fig. 3b).

The latency, localization of optimal stimulation site, histological verification and the abolition of the response by a neuromuscular blocker provide strong evidence that the EMG activity was due to stimulation of cerebellar nucleofugal pathways, involving brainstem descending motor pathways [44, 46].

In summary, the responses evoked by mossy fibre, parallel fibre and climbing fibre activation were remarkably similar to those described previously in vivo [e.g. 30, 42, 47], suggesting that excitatory synaptic inputs to Purkinje cells are preserved in the PHBP. Similarly, the EMG responses evoked by cerebellar nuclear microstimulation suggest that at least part of the cerebellar output to the forelimbs is also preserved in this preparation.

Activity Patterns of Individual Cerebellar Neurones

To further assess cerebellar function in the PHBP we obtained extracellular recordings from single cerebellar neurones in 15 preparations. Electrode penetrations were made orthogonal to the cerebellar cortical surface and spontaneous spike trains were obtained from a total of 50 single units. These were obtained from a wide range of different lobules (including anterior parts of the cerebellum not directly accessible in vivo), and also from a range of medial-lateral positions. No systematic differences in response properties were observed in relation to anatomical location. The stability of the preparation meant that single units could be readily recorded for extended periods of time (when tested, for as long as 3.5 h). All units were spontaneously active. Of the 50 units studied in detail, 25 were identified as Purkinje cells based on the presence of complex spikes in their spike trains (Fig. 4a). Three of the 25 Purkinje cells displayed only complex spikes. Complex spikes had low firing rates, occurring at mean frequencies that ranged in individual Purkinje cells between 0.10–5.15 Hz (Fig. 4 bi, Table 1, overall mean of 0.77±0.20 Hz, n=25 cells). Complex spike firing patterns were highly irregular; as shown by their coefficient of variation (Table 1), and by their range of interspike intervals (Fig. 4 bii; the shortest interval was 7.84 s and the longest was 10.44 s, Table 1). Complex spike autocorrelograms (Fig. 4 biii) revealed no obvious regularity or rhythmicity.

Simple spikes were usually biphasic positive-negative actions potentials with a duration of >0.4 ms (Fig 4a). Simple spike firing patterns were also irregular as shown by interspike interval histograms which were positively skewed (Fig. 4 cii), and also by the coefficient of variation of their interspike intervals (range, 0.26–3.19 ms; mean=0.96 ms±0.14, n=22,
Fig. 4. Purkinje cell single unit recordings. a Recording from a Purkinje cell showing spontaneous complex (dot) and simple spike activity. Lower panel shows ten superimposed complex spikes (left hand panel) and simple spikes (right hand panel). bi Population histogram of mean firing rates of complex spikes \((n=25)\). bii Complex spike interspike interval distribution. biii Autocorrelogram of complex spike spontaneous activity. ci Simple spike interspike interval distribution. cii Population histogram of mean firing rates of simple spikes \((n=22)\). ciii Autocorrelogram of simple spike spontaneous activity. d Sample trace of an intracellular Purkinje cell recording at the level of the proximal dendrites. \(CV\) coefficient of variation.
Table 1). Purkinje cells displayed a wide range of individual firing rates (11–76 Hz, Fig. 4 ci), with an overall mean rate of 42.6±4.2 Hz. The irregularity of simple spike firing was also demonstrated by autocorrelograms (Fig. 4 ciii). Importantly, none of the 22 Purkinje cells studied in detail displayed the rhythmic pacemaker-like pattern of simple spike firing that occurs in the absence of normal background excitatory synaptic input [14, 17]. Although not studied systematically, we were also able to make intracellular recordings from Purkinje cells. For example, Fig. 4d, demonstrates a recording characteristic of Purkinje cell proximal dendritic recordings [15, 48].

A total of 19 units were categorised as cerebellar interneurones, based on their depth in the cerebellar cortex, spike waveform, absence of complex spikes and firing patterns as determined previously in vivo [49, 50]. Putative molecular layer interneurones (n=13) were located in the superficial part of the cerebellar cortex at depths 100–300 μm below the surface including just above the Purkinje cell layer. Typically, they had biphasic positive-negative action potentials >0.8 ms (Table 1; Fig. 5 ai). They fired more slowly and regularly than Purkinje cells, at rates of 1.5–27.5 Hz (Fig. 5 aii; mean firing rate 12.7±2.3 Hz). Their more regular rate of discharge was evident in their interspike interval histograms which tended to be more symmetrical than those of Purkinje cells (Fig. 5 aiii) and their tendency to fire rhythmically was evident by the presence of multiple peaks at regular intervals in their autocorrelograms (Fig. 5 aiv).

Putative Golgi cells (n=6) were located in the granular layer of the cerebellar cortex, below the Purkinje cell layer and had long duration (>1.1 ms) biphasic action potentials (Fig. 5 bi), and a long tuning distance (>100μm electrode movement). In comparison to Purkinje cells, putative Golgi cells exhibited a slower although irregular firing rate (range, 3.7–26.4 Hz; Fig. 5 bii; mean firing rate 12.0±3.3 Hz; Table 1). The more irregular firing rate of putative Golgi cells was evident in their interspike interval histograms which had broader peaks, longer intervals between spikes (Table 1) and a lack of multiple peaks in their autocorrelograms (Fig. 5 biv).

To assess whether stable recordings from cerebellar cortical neurones could be maintained in the PHBP over extended periods without any deterioration in their firing properties, four of the cerebellar neurones (three Purkinje cells and one interneurone) were recorded for a duration of 1.5–3.5 h. No significant difference was found (paired t test, P>0.05) in the mean firing rate and coefficient of variation when the spike trains in the first half of the recording period were compared to those obtained in the final half.

Extracellular recordings were also made of the spontaneous activity of six units in nucleus interpositus. In each
In general, interpositus neurones displayed a highly irregular discharge pattern (coefficient of variation ranged from 0.41–1.94; mean, 1.07±0.25); interspike interval histograms were positively skewed (Fig. 6c; Table 1) and no prominent peaks or troughs were evident in their autocorrelograms (Fig. 6d).

Purkinje cells and interpositus neurones were also found to be responsive to tactile stimulation and electrical stimulation of the periphery. In the example shown in Fig. 7a, light pressure of the periorbital region with a probe resulted in an increase in firing frequency above resting
Fig. 6. Cerebellar nuclear neurones. a Spontaneous activity of a cerebellar interpositus neurone. Lower panel shows ten superimposed spikes at an expanded time base. b Interspike interval histogram of a cerebellar interpositus neurone. c Population histogram of mean firing rates of nuclear neurones (n=6). d Autocorrelogram of spontaneous activity of nuclear neurone.

discharge rates in an interpositus neurone while Fig. 7b shows that electrical stimulation of the facial nerve caused a phasic increase and subsequent phasic decrease in Purkinje cell simple spike activity. Thus, mechanical and electrical peripheral stimuli can be effective at modifying the firing patterns of cerebellar neurones in the PHBP.

Finally, in two experiments we tested the effects of adding harmaline to the perfusate. Harmaline has long been known as a tremogenic agent that acts on the inferior olive [e.g. 51, 52]. Consistent with previous studies in the intact animal, harmaline caused an increase in the frequency of complex spikes (from ∼0.2 Hz to 2.0 Hz), accompanied by the development of a rhythmic pattern of firing, while simple spike activity was abolished (not shown).

Discussion

The present study investigates the use of a novel decerebrate perfused hindbrain and upper body in situ preparation that maintains the cerebellum in relation to its extrinsic connections. Although the cerebro-cerebellar pathway is absent, nevertheless some cerebellar circuits can still be maintained in the absence of this input. Field potential analysis and EMG recordings provided evidence that at least some of the major cerebellar input and output

Fig. 7. Cerebellar neuronal responses to peripheral stimulation. a Peri-event time histogram of the activity of a cerebellar interpositus nuclear neurone in response to mechanical stimulation of the periorbital region. Period of mechanical stimulation is indicated by length of horizontal bar. b Peri-event time histogram of the simple spike activity of a Purkinje cell in response to electrical stimulation of the facial nerve. Stimulus onset=time 0. Bin size=10 ms.
Comparison with Physiological Studies In vivo and In vitro

Extracellular recordings of Purkinje cell simple spike activity, putative Golgi cells and molecular layer interneurons indicated that such cells were tonically active in the PHBP. Average firing rates, spike widths, interspike intervals and coefficients of variation were all within the ranges reported by others in anaesthetised rats [53–56], as well as awake rats [57, 58] and cats [59, 60]. For example, simple spike activity of Purkinje cells in anaesthetised and awake animals at rest exhibit mean firing rates of 40–50 Hz while the mean firing rate found in the PHBP was ~45 Hz. Likewise, molecular layer interneurones and Golgi cells exhibit mean firing rates of 10–30 Hz [56, 61] and 2–25 Hz, respectively [49, 50, 56, 60] which compares favourably to the mean rates found in the PHBP (~13 Hz for molecular layer interneurones and ~11 Hz for putative Golgi cells).

Similarly, spontaneous firing rates of cerebellar nuclear neurons have been reported to be 34 Hz in the awake cat [59], while in the PHBP the corresponding value is 32 Hz. However, this value is lower than the rate of 55 Hz reported in P50–100 day anaesthetised rats [54] and the rate of 43 Hz reported in P20–26 day awake rats [62]. The lower mean firing rate in the PHBP may be due to the lack of excitatory drive from nuclear collateral inputs arising from cerebro-ponto-cerebellar projections [e.g. 63] and/or the lower temperature (32–34°C) used to maintain the PHBP, as dissociated cerebellar nuclear cells maintained at 31°C discharge at approximately 20 Hz [64].

The majority (~85%) of Purkinje cells recorded in the PHBP discharged complex spikes at <1.0 Hz. This is consistent with studies in ketamine/xylazine anaesthetised rats [53], and awake mice [65], but is somewhat lower than reported in other in vivo preparations (e.g. 1.5 Hz, [61], 1.52 Hz, [66], 1.34 Hz, [56]). An important consideration is decerebration which may have affected complex spike firing rates. In decerebrate animals complex spikes were found to be detectable in only one third of Purkinje cells [32, 36], and lower mean complex spike firing rates have been reported in the decerebrate ferret (0.48 Hz, [67]) and cat (0.67 Hz, [68]). Nevertheless, it appears that when the climbing fibre system is being driven in the PHBP, as was the case in the climbing field potential mapping and in the harmaline experiments, the latency and patterns of responses closely matched those reported in intact preparations (e.g. [28, 69, 70]).

Table 2  Advantages and disadvantages of the PHBP preparation

| ADVANTAGES                                                                 | DISADVANTAGES                                                                 |
|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Allows access to parts of rodent cerebellum not accessible in vivo (in both decerebrate and anaesthetised preparations), notably the anterior lobe. | The main limitation is that the PHBP is decerebrate. This will deprive the cerebellum of a major source of input. If required, anaesthesia can be used as an alternative to decerebration |
| Unlike in vitro preparations, the PHBP retains functional connectivity between the cerebellum, brainstem and the upper body and head (and can be modified to include the whole body if required) | Young age of rats (P45). However it should be noted that the animals are older than those frequently used in in vitro studies |
| The high mechanical stability combined with increased accessibility allows for long term recording/stimulation of multiple hindbrain sites | Cerebellar anatomical landmarks are less clear as major blood vessels such as the paravermal vein are transparent. However, dye can be introduced into the perfusate to temporarily mark blood vessels |
| Avoids the complications of anaesthesia | Viability is limited to about 5 h |
| Allows greater control over pharmacological manipulations as drugs can be rapidly introduced and washed out, and neurotoxins can be used that would be lethal in vivo | Peripheral electrical stimulation and recording (e.g. of nerves) is more complicated than in vivo because of the perfusate; electrical contacts need to be made water tight |
| In contrast to in vitro preparations the PHBP allows the study of a range of different sensory modalities, including: cutaneous and proprioceptive pathways, and whisker sensory inputs to hindbrain circuits | The thorax and upper forelimbs are usually skinned to prevent clogging of tubing/filters with hair and debris. This will alter sensory inputs but regions of skin of interest can be left intact e.g. the face (including whiskers) and distal forelimbs |
| The PHBP is quicker and easier to set up than in vitro preparations |                                                                                                                                                               |
intact preparation may be beneficial when studying the parallel fibre system.

Advantages and Disadvantages of the PHBP

Table 2 summarises the advantages and disadvantages of the PHBP. By comparison to studies in anaesthetised animals the PHBP has a number of advantages: (1) Cerebellar circuitry can be studied without the complication of anaesthetics, which are known to have a profound effect on transmission of sensory-evoked responses [37, 71]. (2) the PHBP, like previous ‘block’ preparations [21, 22, 24–27], allows direct access to areas of the cerebellum (and brainstem) that are difficult to reach in vivo, such as rostral parts of the cerebellar anterior lobe, paraflocculus and flocculus. (3) Also by contrast to in vivo preparations, respiratory and heart movements can be eliminated if necessary, so stable recordings are possible which can be maintained for extended periods of time (in the case of extracellular recordings for several hours if necessary) with no detectable change in firing properties.

By comparison to in vitro brain slice preparations, an additional important feature of the PHBP is that it also preserves hindbrain connections with the head and forelimbs. The latter is particularly useful when examining cerebellar pathways involved in the control of forelimb movements, as nucleus interpositus projections to the cerebral spinal cord and forelimb muscles appear to function as normal in this preparation. Also in contrast to slice preparations, the PHBP is relatively quick to set up; approximately 30 min, whereas slice preparations can take up to 2 h. However, the length of the recording period in the PHBP is highly dependent on the adequacy of perfusion and how rapidly perfusion is initiated. For this purpose, monitoring of phrenic nerve activity is essential as the neurogram is a reliable indicator of the general viability of the preparation. On occasions when phrenic activity was abnormal (tonic or apneic), little or no neuronal activity could be obtained from the cerebellum. In preparations with adequate perfusion, recordings are possible for up to 5 h. After this time, recording quality rapidly declines in parallel with general deterioration of the preparation as determined by a change in phrenic nerve activity from regular ramp-like bursts to irregular activity.

In terms of limitations, the young age of rats (P45) used for the preparation is a potential concern. Morphological and functional maturation of rat mossy fibre-granule cell synapses occurs later (P60) [72, 73]. However, a viable preparation is critically dependent on adequate perfusion pressure. This precludes the use of adult animals due to the substantial increase in perfusion flow rate required in order to achieve sufficient perfusion of the vascular tree as well as the increased risk of hypoxic insult during preparation. Attempts to increase flow rates in larger animals by increasing the pump flow rates were unsuccessful, because phrenic nerve activity becomes abnormal (exhibits tonic activity) and the preparation rapidly becomes oedematous. Therefore for a viable preparation, younger animals need to be used in comparison to those normally used for in vivo studies. Nevertheless, the upper age limit of the animals (P35–45) is older than those used for some in vitro investigations (P11–25, e.g. [74–76]).

Other limitations of the PHBP include difficulty in identifying different cerebellar regions due to the lack of visible surface blood vessels as anatomical landmarks, and the lack of skin inputs. However, regions of skin can be left intact and the preparation can include the whole body if required. A further and arguably the most important limitation is the lack of cerebro-cerebellar projections. Although Purkinje cell simple spikes could be activated by peripheral stimulation, we were not able to record longer latency field potential responses attributed to cerebral cortical inputs, most likely due to the decerebration [77–79]. Note however that decerebrate preparations can be used to study some aspects of cerebellar contributions to a range of behavioural responses such as eye blink conditioning (for e.g. [45, 80, 81]); but see [82] and motor control (for e.g. [83–85]). Also, if descending pathways are to be studied, then the preparation can be anaesthetised through the addition of anaesthetics to the perfusate whilst leaving the cerebral cortex intact [86].

In conclusion, the present study provides evidence that the cerebellum and some of its major interconnections with the rest of the nervous system remain operational in the PHBP. The PHBP should therefore be a useful complement to in vivo and in vitro models for studies requiring stable recordings of single neurones over several hours (including simultaneous recordings), whilst retaining interconnections with other neuronal networks, such as those involved in the control of forelimb movements.

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