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In vitro electrophysiological recordings of multiple brain regions from aged pig tailed Macaques

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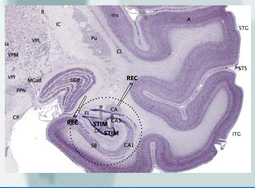
INTRODUCTION

The relatively long lifespan, extended infancy and social cognition of non-human primates (NHPs) parallels many aspects of human development and the physiological, behavioural, and neuroanatomical similarities to humans can facilitate translation of therapeutic strategies to a range of human conditions. To date, the vast majority of electrophysiological recordings performed in NHPs have been achieved in juvenile animals with little if any work performed in aged animals.

METHODS

Data was obtained from two aged female Macaque nemestrina monkeys. Both animals were scheduled for euthanasia due to significant health issues and were obtained from the Monash University breeding facility with all work conducted under scavenging ethics and approved by the Institutional (Monash University) Animal Ethics Committee and conform to NHMRC policies on the use of animals in neuroscience research. Animals were maintained under deep anaesthesia while a craniotomy was conducted to expose the brain. Following the craniotomy, animals were perfused with ice-cold, oxygenated artificial cerebrospinal fluid ((in mM): NaCl 127, KCl 5, KH_2PO_4 3, $MgCl_2$ 2, $CaCl_2$ 2, $NaHCO_3$ 23 and glucose 10) to displace blood and to reduce the temperature of the entire brain. The brain was removed within minutes of death and specific brain regions were immediately dissected for subsequent *in vitro* electrophysiology. Brain slices of 400 µm thickness were cut in chilled (<4°C) artificial cerebrospinal fluid (aCSF) using Leica VT1000s microtomes.

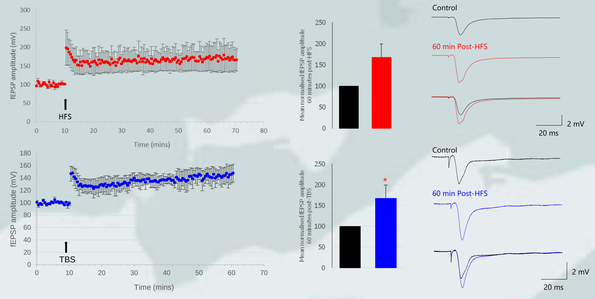
Slices were maintained in oxygenated (95% O_2 / 5% CO_2) aCSF at room temperature for at least 1 hr prior to electrophysiological recording, aCSF composition (mM): NaCl 127, KCl 1.6, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26 and glucose 10. Whole-cell patch clamp and extracellular recordings were performed from varying brain regions using Axopatch 1D, Multiclamp 700A, Axoclamp 2A and CED Neurolog Amplifiers. For LTP experiments, Schaffer collaterals were stimulated with a concentric bipolar electrode and the evoked fEPSP recorded from the stratum radiatum of the CA1 region of the hippocampus with stimulation parameters set to produce a fEPSP of approximately 50% of maximum. Effects of high-frequency stimulation (HFS, 100 Hz, 1 sec) and Theta Burst stimulation (TBS) : 10 trains of 4 pulses, 100 Hz, 200 ms apart were examined. Whole-cell patch clamp experiments were performed at room temperature using an intracellular solution composed of 140 Potassium gluconate; 10 KCl; 1 EGTA-Na; 10 HEPES; 4 Na_2ATP; 0.3 Na_2GTP, osmolarity and pH adjusted with sucrose and KOH, respectively. A concentric bipolar electrode was used to stimulate synaptic inputs. For extracellular Isolated Sciatic Nerve (ISN) experiments, both sciatic nerve were surgically isolated, split into smaller fasciculi and recorded in a custom built chamber. Compounds were bath applied for all techniques.





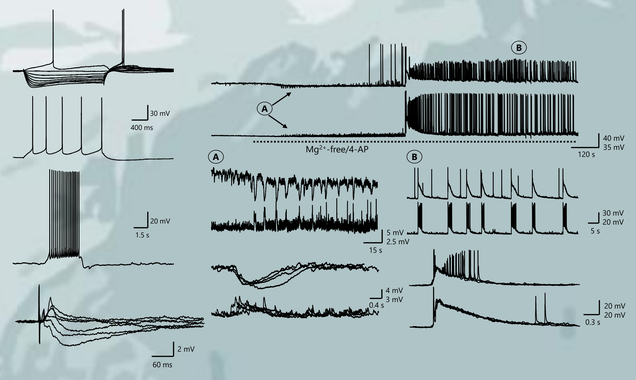
RESULTS

❶ HIPPOCAMPAL LONG-TERM POTENTIATION



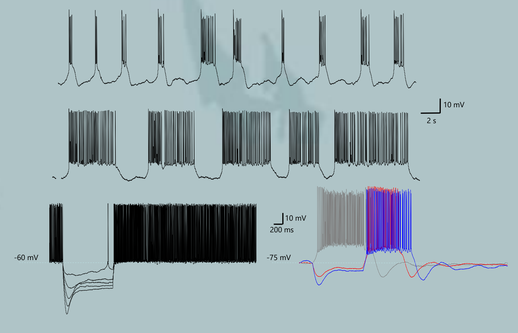
Example plots demonstrating the level of potentiation of fEPSPs in hippocampal acute slices following high frequency stimulation (HFS, 100 Hz, 1 sec) or Theta-burst stimulation (10xTBS) of the schaffer collateral pathway.

❷ HIPPOCAMPUS



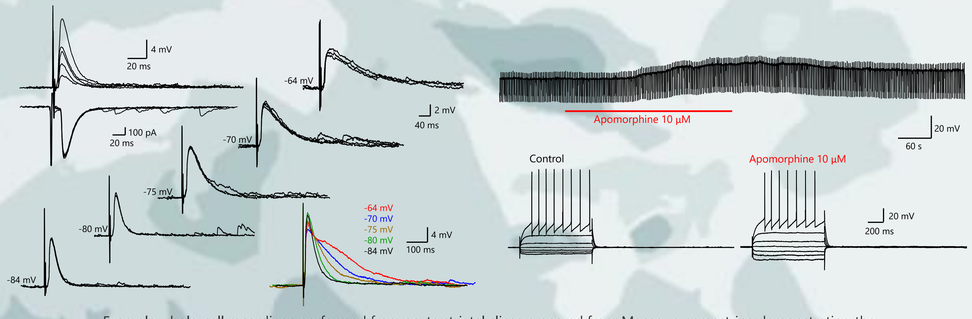
Example whole-cell recordings performed from acute hippocampal slices prepared from Macaque nemestrina demonstrating the electrophysiological characteristics of hippocampal pyramidal neurones.

❸ CEREBELLUM



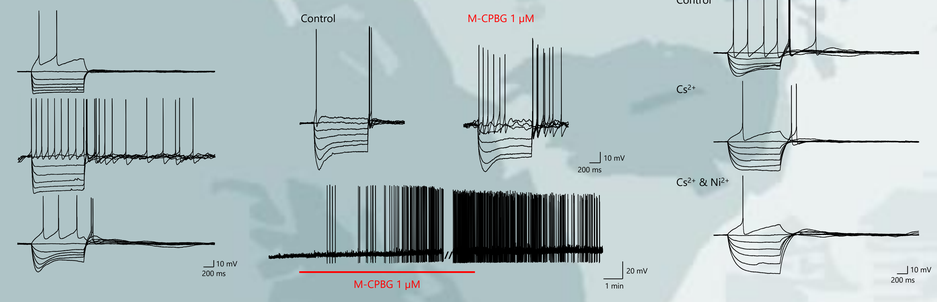
Example of a whole-cell recording performed from acute cerebellar slices prepared from Macaque nemestrina demonstrating the characteristic firing patterns and electrophysiological properties of a Purkinje neurone.

❹ STRIATUM



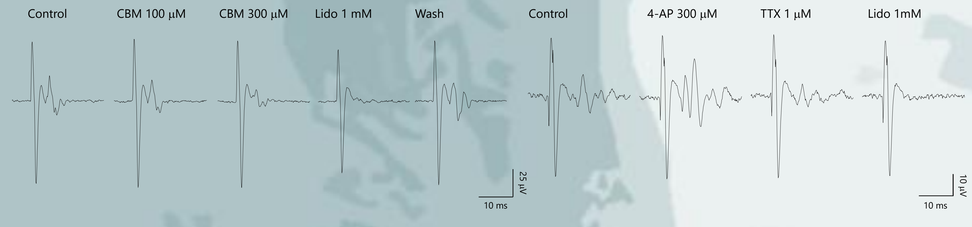
Example whole-cell recordings performed from acute striatal slices prepared from Macaque nemestrina demonstrating the electrophysiological characteristics of Medium Spiny Neurones (MSNs) and their response to application of Apomorphine.

❺ PREFRONTAL CORTEX



Example whole-cell recordings performed from acute cortical slices prepared from Macaque nemestrina demonstrating the electrophysiological characteristics of prefrontal cortical neurones, their response to application of the 5-HT_3 receptor ligand M-CPBG, and the active conductances expressed by these neurones as revealed by application of Caesium and Nickel.

❻ ISOLATED SCIATIC NERVE



Example extracellular recordings performed from acutely isolated sciatic nerve preparations demonstrating the effects of common pharmacological tools on compound action potentials (CAP)

CONCLUSIONS

This approach enabled *in vitro* brain slice preparations to be maintained viable for at least 36 hours with the intrinsic, electrophysiological, synaptic and pharmacological properties of neurones characterised over this period. Our data show the viability of recording *in vitro* in aged NHP and highlights the potential of such studies for future translational studies, maximising the opportunity for NHP tissue use whilst minimising animal suffering.

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