Electrophysiological characterization and MCH/orexin neuronal distribution in the lateral hypothalamus of naked mole-rats (*Heterocephalus glaber*)

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Abstract

The lateral hypothalamus (LH) controls various homeostatic processes, including sleep-wake cycles, energy balance and thermoregulation in many mammalian species. In the LH, melanin-concentrating hormone (MCH) and hypocretin/orexin (HO) containing neurons differentially regulate these processes. Naked mole-rats (NMR) (*Heterocephalus glaber*) are eusocial mammals with remarkable physiological peculiarities including extreme longevity without significant weight gain and absence of thermoregulation. Altered hypothalamic function could potentially underlie the unusual NMR phenotypes, but to date electrophysiological characterization of LH NMR neurons and expression of MCH and HO is missing. Here, we performed whole-cell recordings from LH neurons in acute NMR and mouse brain slices and found that NMR and mouse neuronal basic properties and activities were comparable. Additionally, we showed that both MCH-positive and HO-positive neuronal populations exist in the NMR hypothalamus and although the majority of MCH- and HO-positive neurons are located in the LH, as previously described in rodents, significant differences exist in MCH/HO distribution in other NMR hypothalamic areas. These results indicate that NMR LH neurons are comparable to mouse neurons with respect to their electrophysiological properties, but differences in the neuronal MCH and HO populations in hypothalamic regions exist and may contribute to the adaptive changes seen in NMR homeostatic processes.

Key words

Lateral hypothalamus, naked mole-rat, melanin-concentrating hormone, hypocretin/orexin-A

Abbreviation

Ant H, Anterior hypothalamus; AP, action potential; cp, cerebellar peduncle; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; MCH, melanin-concentrating hormone; NMR, naked mole-rat; opt., optical tract; ParaV, paraventricular nucleus; VMH, ventromedial hypothalamus; ZI, zona incerta.

1. Background

The hypothalamus is an important brain region for regulating homeostatic processes including appetite [1], sleep-wake cycles [2], thermoregulation [3], energy balance [4,5] and breathing [6,7]. The lateral hypothalamus (LH) is a key hypothalamic subregion that modulates

these homeostatic processes, two key neuropeptides involved being melanin-concentrating hormone (MCH) and hypocretin/orexin (HO) [8].

Naked mole-rats (NMRs, *Heterocephalus glaber*) are subterranean, eusocial rodents [9,10] displaying numerous physiological peculiarities [11], including extreme longevity [12], insensitivity to certain noxious/irritant stimuli [13,14] and hypoxia/hypercapnia resistance [15,16]. Interestingly, numerous characteristics of NMR physiology suggest differences in hypothalamic activity. Firstly, only one female per colony breeds, other animals being in a prepubescent state [17]. In addition, although NMRs can live for up to 30 years, they do not gain significant weight with ageing [18], have a low resting metabolic rate [19], poor thermoregulatory capacity [19,20] and lack a clear circadian rhythm [21,22]. Although research has been conducted on hypothalamic function in the NMR with regard to animal social status [23], no one has yet investigated LH neuronal electrophysiological properties or expression of MCH and HO.

Here, we investigated the basic electrophysiological properties of unidentified hypothalamic neurons in NMRs compared to mice, using whole cell recording methods in acute brain slices and immunohistochemistry to determine the expression of MCH and HO in the LH.

2. Methods

(a) Electrophysiology

Coronal slices were made from adult C57BL6 mice and NMRs, respectively. Brain acute slices were prepared and electrophysiological recordings performed as previously described [24]. Whole-cell recordings were performed at 35°C using an EPC-10 amplifier and Patch-Master software (HEKA Elektronik). Data were analyzed with Axograph software and statistical analysis were done using Prism (GraphPad). Unpaired two-sided t-tests were used to compare electrophysiological parameters between mouse and NMR neurons (* $p = 0.01$, **** $p < 0.0001$). Values represented as mean ± SEM.

(b) Immunohistochemistry and image analysis

Brain slices from mice and NMRs were prepared for immunolabelling as previously described [24]. 40 µm thick coronal sections were incubated overnight with primary antibodies (rabbit anti-HO cat. n° H-003-30, rabbit anti-MCH cat. n° H-070-47, (Phoenix pharmaceuticals), 1:1000 and 1:1500, respectively), washed before incubation with the secondary antibodies (anti-rabbit Alexa 488, Invitrogen, 1:1000) and counterstained for Nissl following manufacturer's instructions (NeuroTrace 530/615, Molecular Probes). Images were acquired with a Leica confocal microscope. Hypothalamic subregions were delineated using mouse/NMR brain atlases [25,26]. Immunoreactive neurons were counted manually using the ImageJ cell counter plugin. Two mice

and two NMRs were used (n = 16 mouse sections for MCH and HO; n = 21/24 NMR sections for MCH/HO, respectively). Data were compared using unpaired one-way ANOVA tests, Tukey's multiple comparison post hoc test (****, $p < 0.0001$) with Prism. Values represented as mean ± SEM.

3. Results

(a) Basic electrophysiological properties of LH NMR neurons

Patch-clamp recordings were performed in a neurochemically-blind manner in NMR and mouse LH slices (Figure 1A(a-b)). Similar spiking patterns in response to current injections were seen in both species (Figure 1B(a-b)), suggesting similar heterogeneity among LH neurons. Spontaneously and non-spontaneously spiking neurons were observed in both mouse and NMR LHs, as well as neurons expressing inwardly rectifying currents (I_h), neurons with large access resistance and neurons with a bursting phenotype. Quantitative analysis showed no differences between the resting membrane potentials (NMR: -64.6 ± 1.7 mV; mouse : -62.2 ± 1.8 mV; $p = 0.33$) and access resistances (NMR: 223.8 ± 19.1 mΩ; mouse: 174.9 ± 1.4 mΩ; $p = 0.09$) between mouse (n = 28) and NMR (n = 33) LH neurons (Figure 1C). Evoked action potentials (AP) were found to have a similar rise times (NMR: 1.7 ± 0.2 ms; mouse: 2.3 ± 0.5 ms; $p = 0.25$) while evoked APs were greater in height (NMR: 40.0 ± 1.7 mV; mouse: 34.0 ± 1.4 mV; * $p = 0.01$) and half-width (NMR: 1.26 ± 0.02 ms; mouse: 0.98 ± 0.06 ms; **** $p < 0.0001$) in recorded NMR LH neurons compared to mouse (Figure 1C).

(b) Expression of MCH and HO neurons in NMR and mouse LHs

We next characterized the distribution of MCH and HO neuronal populations in the NMR and mouse hypothalami (Figure 2A-B). In both species, the vast majority of HO-positive neurons were found in the LH, although in mice the rest was found only in the dorsomedial hypothalamus (DMH) while NMR HO-positive neurons were scattered in the adjacent regions (Figure 2C). In contrast, of those MCH-positive neurons, a greater proportion was distributed in the NMR LH (95.1%) than in the mouse LH (78.8%), with mouse MCH-positive neurons also being observed in the DMH (8.3%) and zona incerta (ZI, 12%) (Figure 2D).

The density of HO-positive neurons was lower in NMR LH compared to mouse LH (Figure 2E) (1202 ± 179.4 cells/mm^3 in mouse, 461.1 ± 43.24 cells/mm^3 in NMR; $p < 0.0001$), whereas in surrounding areas, densities were comparable between species. In contrast, MCH-positive neuron densities were similar in NMR and mouse LHs (Figure 2F) (1453 ± 153.3 cells/mm^3 in mouse, 1327 ± 103.4 cells/mm^3 in NMR), but MCH-positive neuron densities in the ZI and DMH

were lower in NMR compared to mouse (ZI: 1356 ± 147.4 cells/mm^3 in mouse, 101.6 ± 25.57 cells/mm^3 in NMR; DMH: 1050 ± 190 cells/mm^3 in mouse, 196 ± 33.6 cells/mm^3 in NMR, $p < 0.0001$).

4. Discussion

In this study, we observed that NMR and mouse LH neurons were broadly similar with respect to their spiking patterns, resting membrane potentials, input resistance and AP height (Figure 1). However, AP widths were shorter in NMR LH neurons compared to mouse neurons and in comparison to mouse LH neurons the AP rise time was longer in NMR LH neurons, the former possibly enabling higher firing frequency. Although informative to know if AP differences occur in particular LH neuronal subpopulations, the transgenic reporter lines that can be utilized for this purpose in mice [27,28] are absent for NMR analysis.

We further showed that in NMRs, the majority of MCH-positive and HO-positive neurons are located in the LH, as in other rodents [29,30]. However, HO-positive neurons were less numerous in the NMR LH compared to mouse. By contrast, MCH-positive neuron densities were similar in the LH of NMR and mouse, but were smaller for NMR in ZI and DMH compared to mouse. The physiological significance of such differences is not known and additional research is needed to further investigate the role of HO and MCH neurons in NMR physiology and whether these differences perhaps contribute to the unusual phenotypes of the NMR that are linked to hypothalamic function.

Figure Legends

Figure 1: Electrophysiological properties of mouse and NMR LH neurons. A. Neurochemically-blinded patch clamp recordings were made from mouse (a) and NMR (b) LH neurons at the locations depicted by black stars. Coordinates are given in millimeters from Bregma. B. Recordings of mouse (blue traces) and NMR (red traces) neurons were performed while injecting 1 second steps of current (black traces) in current clamp configuration, and allowed identification of several types of neurons similarly found in both species. C. Electrophysiological parameters of mouse (blue bars) and NMR (red bars) LH neurons (unpaired two sided t-tests, * $p = 0.01$, **** $p < 0.0001$).

Figure 2: MCH and HO neuronal populations in NMR and mouse hypothalami. Coronal sections of mouse and NMR brains stained against HO (A) and MCH (B). Dashed line indicated the midline between hemispheres. Insets shows magnification of positively labelled cells. Scale bars: A-B,

500 µm; insets, 100 µm. C-D. Distribution of the HO-positive (C) and MCH-positive (D) neurons within the different hypothalamic nuclei of mouse and NMR brains. E-F. Density of labelled neurons (E for HO; F for MCH) per mm^3 of NMR (light bars) and mouse (opaque bars) hypothalamic nuclei. One-way ANOVA test; Tukey's multiple comparison test, ****, $p < 0.0001$.

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Author contributions

ZH, JAS and ESS designed the study. JAS performed/analyzed the patch-clamp experiments. ZH performed/analyzed the immunohistochemistry experiments. All authors wrote the manuscript.

Data accessibility

The datasets supporting the conclusions of this article are available in the University of Cambridge https://doi.org/10.17863/CAM.21892.

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Competing Interests

The authors have no competing interests.

Ethical statement

All experiments were conducted in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986 Amendment Regulations 2012 under a Project License (70/7705) granted to E. St. J. S. by the Home Office; the University of Cambridge Animal Welfare Ethical Review Body also approved procedures.

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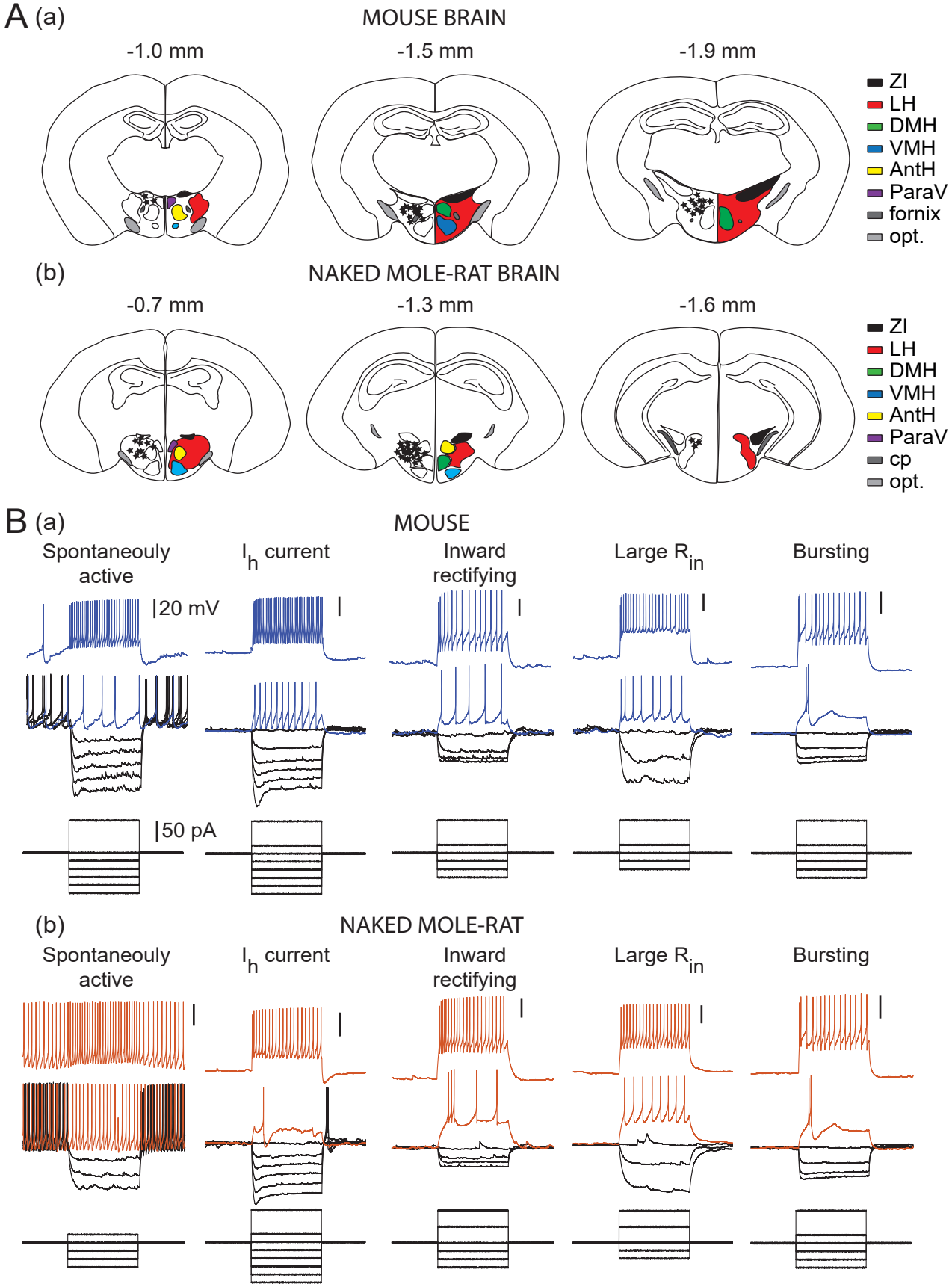
A (a)
MOUSE BRAIN
-1.0 mm
-1.5 mm
-1.9 mm
ZI
LH
DMH
VMH
AntH
ParaV
fornix
opt.
(b)
NAKED MOLE-RAT BRAIN
-0.7 mm
-1.3 mm
-1.6 mm
ZI
LH
DMH
VMH
AntH
ParaV
cp
opt.
B (a)
MOUSE
Spontaneouly active
I_h current
Inward rectifying
Large R_{in}
Bursting
20 mV
50 pA
(b)
NAKED MOLE-RAT
Spontaneouly active
I_h current
Inward rectifying
Large R_{in}
Bursting
C
Mouse
NMR
Resting potential (mV)
-40
-50
-60
-70
Input resistance (mOhms)
300
200
100
0
Action Potential Height (mV)
40
30
20
10
0
Action Potential Half-Width (ms)
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0.8
0.4
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Action Potential Risetime (ms)
3
2
1
0
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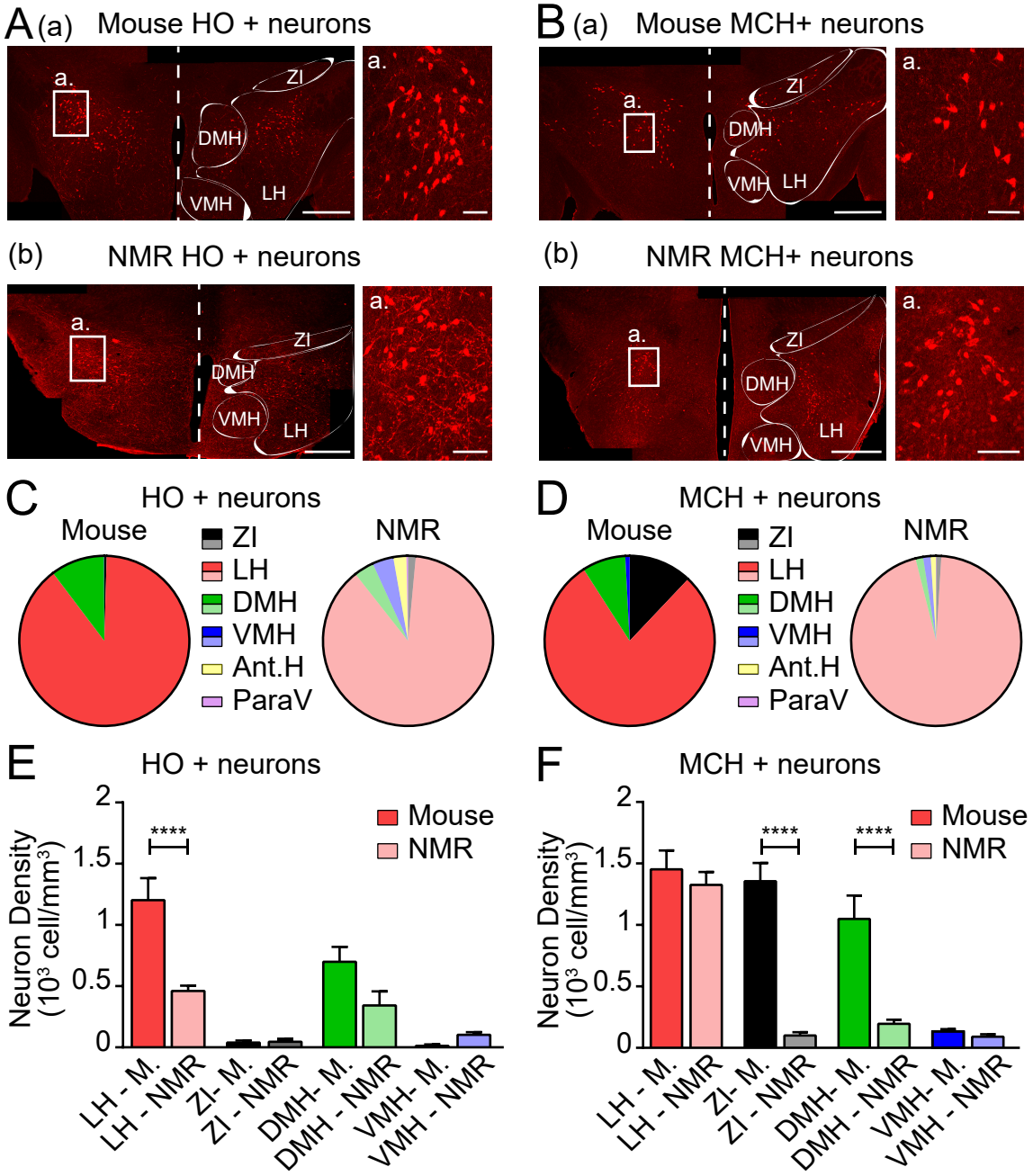
FIGURE 2


A (a) Mouse HO + neurons
a.
ZI
DMH
VMH
LH
(b) NMR HO + neurons
B (a) Mouse MCH+ neurons
(b) NMR MCH+ neurons
C HO + neurons
Mouse
NMR
ZI
LH
DMH
VMH
Ant.H
ParaV
D MCH + neurons
E HO + neurons
Neuron Density (10^3 cell/mm^3)
Mouse
NMR
****
LH - M.
LH - NMR
ZI- M.
ZI - NMR
DMH- M.
DMH - NMR
VMH- M.
VMH - NMR
F MCH + neurons