Safe prolongation of pharmacologically induced hypothermia for biomedical applications and space exploration

Torpor in hibernating animals is characterized by an active reduction in metabolic rate, body temperature and brain activity (Nedergaard and Cannon, 1990). Research interest in replicating torpor, in non-hibernating mammals and ultimately into human, is constantly increasing due to the uses into a variety of biomedical applications (Cerri et al., 2013; Tupone et al., 2013a, b; Tupone and Morrison, 2014; Tupone et al., 2016; Cerri, 2017).

Therapeutic hypothermia and the positive outcome that hypothermia contributes to brain and cardiac protection following ischemia is one of the main reason stimulating this research (Tupone and Morrison, 2014; Drew et al., 2015; Tupone et al., 2016).

Furthermore, an effective procedure to mimic torpor could be very beneficial for the human exploration of the solar system counteracting some of the most adverse effects induced by long term permanence in microgravity, including radiation exposure (McGee-Lawrence et al., 2008; Cerri et al., 2016), and, as well, for the use of animals in space (Griko and Regan, 2018).

Recently, varieties of approaches have been proposed to induce a torpor like state in non-hibernating animal (Blackstone et al., 2005; Cerri et al., 2013; Tupone et al., 2013a; Dugbarney et al., 2015; Cerri et al., 2017). However a systematic study, assessing the length of the permanence in this state as well as the recovery to normal physiological and behavioral function has never been performed.

The main aim of this study is to determine: 1) the extension of torpor like state and 2) if the physiological and behavioral performance of these animals are completely recovered to the normal condition after a long term torpor-like state.

We will employ two of our mastered methodology to induce torpor like state: central administration of A1AR agonist (CHA) (Tupone et al., 2013a) or direct inhibition of raphe pallidus (RPa) (Cerri et al., 2013).

The results of this study will allow us to explore the possibility to prolong and help the management of hypothermia/torpor state in non-hibernating animal. This will ultimately be beneficial to acquire more in formation relative to ongoing projects and collaborations, exploring outcomes in ischemic pathologies, perinatal hypoxic encephalopathy, COX inhibitor-resistant fevers and hypothermia-induced radioprotection.

Experimental procedures.

For all the experiments rats will be implanted under general anesthesia with isoflurane with a microcanulla in the lateral ventricle or within the RPa, electrodes for EEG, EMG, diaphragmatic EMG, core temperature probe and AP telemetric transmitter. Two weeks recovery after implantation surgery will be allowed. Following the recovery, rats, tethered to the acquisition system for the recording of the physiological variables, will be maintained in a thermoregulated and sound-attenuated chamber kept at 25°C. To evaluate the degree of recovery following pharmacological induction of torpor, sleep homeostasis, physiological variables dynamics during specific states of the sleep-wake cycle will be analyzed and compared before and after hypothermia treatment. Sleep stages, will be visually scored by an operator and EEG power spectrum will be calculated. Circadian and ultradian sleep rhythms and ultradian basic-rest activity cycle will also be evaluated. Rats will also be tested for motor coordination using a Rotarod before and at the recovery from torpor like state.

AIM 1

1. Assess the average hypothermic duration in response to a single central injection of A1AR agonist or direct inhibition of RPa.
   - Rats injected with saline ICV (Hypothermia SAL)
   - Rats injected with CHA ICV (Hypothermia CHA)
2. Assess the possibility of prolonging hypothermia with multiple central injection of A1AR agonist or direct inhibition of RPa.
   - Rats treated with multiple injection of saline ICV (Hypothermia SAL)
   - Rats treated with multiple injection of CHA ICV (Hypothermia CHA)

**AIM 2**

1. Assessment of the physiological functions following prolonged hypothermia.
   - Rats injected with saline ICV (Hypothermia SAL)
   - Rats injected with CHA ICV (Hypothermia CHA)
   - Rats treated with multiple injection of saline ICV (Hypothermia SAL)
   - Rats treated with multiple injection of CHA ICV (Hypothermia CHA)

2. Assessment of behavioral function following prolonged hypothermia.
   - Rats injected with saline ICV (Hypothermia SAL)
   - Rats injected with CHA ICV (Hypothermia CHA)
   - Rats treated with multiple injection of saline ICV (Hypothermia SAL)
   - Rats treated with multiple injection of CHA ICV (Hypothermia CHA)

Tupone D, Morrison S (2014) Hypothermia, torpor and the fundamental importance of understanding the central control of thermoregulation. Temperature (Austin) 1:89-91.
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Allegato 2: PROGRAMMA DI ATTIVITA’ DELL’ASSEGNISTA

The activity of the postdoc will consist in:

i) microsurgery in anesthetized rats including:
   - cannulation of femoral artery and vein
   - cranial surgery for brain transection and drugs microinjection procedures
   - implantation of electrodes for the recording of EMG and probes for the acquisition of skin core and BAT temperature

ii) acquisition, analysis and statistical assessment of the data

The methods in the proposal are well established by the PI as demonstrated by the published works (Madden et al., 2013; Tupone et al., 2013; Roeder et al., 2016; Tupone et al., 2017). The researcher will have constant interaction with the PI who will train the researcher to execute and analyze the data for all the experiments lineup in the proposal. The researcher will also participate to journal clubs in which he will have the opportunity to train is speaker’s ability by presenting and discuss the results of the proposal with other faculty in the department.

A single person can run eight physiological experiment/month. For all the experiments, including a 10% failure rate, we will need a total of 48 rats and 6 months estimated maximum time for the execution of the experiments (see also table 1). Four/five months are required for the analysis of the data. Some of these experiments and data analysis, will be executed by the PI of the project as a training section for the researcher. This will contribute to reduce the work days of the researcher making this project completely suited to be to be executed in one year.

Table 1

<table>
<thead>
<tr>
<th>Specific Aim</th>
<th>Expt</th>
<th>Number of rat/group</th>
<th>Estimated months of work (including failure rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2 groups, 8 rats/group</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>4 groups, 8 rats</td>
<td>4</td>
</tr>
</tbody>
</table>

References


