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Temporary inactivation of the rodent hippocampus: An evaluation of the current methodology

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HIGHLIGHTS

- Investigation of the current methods of temporary inactivation.
- A literature review revealed a variety of methods used for temporary inactivation of the HPC in rats.
- One bilateral infusion site in dorsal HPC does not inactivate the entire structure.
- Needles protruding below the guide cannula cause activation of surrounding neurons.
- Ropivacaine can suppress HPC activity by 83% 45 min after infusion.

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ABSTRACT

Temporary cellular inactivation is a useful and increasingly popular approach in examining brain function. In general the methods allow for fast-acting manipulations that have the advantage of being reversible. However, there is significant variation in detailed procedures across experiments and most authors show very little evidence about the extent or duration of inactivation. Here we investigate a commonly used method of temporarily inactivating the hippocampus in rats. Using immediate early gene activation after electroconvulsive shock we measure the extent of inactivation using different lengths of infusion needles and one vs. two bilateral infusion sites. Our methods allowed us to uncover some possible confounding factors. We suggest specific variations in the procedures which decrease or eliminate these problems. We also investigate the properties of the sodium channel blocker ropivacaine and recommend this drug based on its functional profile and established low level of toxicity.

1. Introduction

Following Paul Broca's report of localization of speech to a region of cerebral cortex in 1861, the study of human patients with damage to one or more parts of the brain has been an important tool in investigating brain function (Kolb and Whishaw, 2008). This approach has been experimentally extended to rodent models where factors such as pre-injury condition, location and extent of damage can be better controlled. However, the protracted time-course from surgery to postsurgical recovery, the possibility with excitotoxins of developing seizures, and compensation from damage (Lomber, 1999), as well as the obvious large drawback – that the damage is indeed permanent – has led to the development of other temporary lesion methods.

Avis and Carlton (1968) demonstrated that by injecting potassium chloride into the brain of a rat, amnesia was observed. Following this finding, temporarily inactivating hippocampus (HPC) has become a powerful tool in the study of the neurobiology of learning and memory. A literature search conducted on June 6th 2011 using the keywords "hippocampus AND inactivation" through Web of Knowledge – Web of Science (http://0apps.webofknowledge.com.darius.uleth.ca) revealed a total of 65 articles published between the years 2001 and 2011 which used temporary inactivation of the HPC in awake rats as a part of their methodology. However, the details of the methods varied greatly (see Table 1 for details).

Several factors in temporary inactivation methods allow for control of the length of inactivation, whether or not fibers of passage are affected, and spread of inactivation, as well as eliminating many

Abbreviations: dHPC, dorsal hippocampus; ROP, ropivacaine; vHPC, ventral hippocampus.

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Table 1
The articles included in the literature evaluation of current methodology used in studies where temporally inactivating the HPC is a part of the methodology. Highlighted is the part of the HPC which the researchers acclaim their experimental findings to, the number of infusion sites used (1 indicates unilateral, 2 indicated bilateral, etc.) the method used to evaluate the extent of the inactivation, drug used, time interval between drug infusion and behavioral testing, as well as the length of the infusion needle. Complete references are provided in the reference section. (Sub: subiculum).

Reference	Described affected structure	Number of infusion sites	Evaluation of inactivation	Drug	Drug infusion – behavioral testing interval	Length of infusion needle
Holahan and Routtenberg (2011)	CA3 of dorsal HPC	2	Cannulae placement	Lidocaine	Post	+1 mm
Telensky et al. (2011)	HPC	2	Ink injection	TTX	40 min pre	+1 mm
Cimadevilla et al. (2011)	HPC	2	Cannulae placement	TTX	20 min pre	+2 mm
Lasseter et al. (2010)	vHPC, DG or pDH	2	Cannulae placement	Baclofen + muscimol	N/A	+1 mm
Parsons and Otto (2010)	dHPC	2	Cannulae placement	Muscimol	30 min pre	+1 mm
McEown and Treit (2010)	dHPC or vHPC	2	Cannulae placement	Muscimol	10 min pre	N/A
McDonald et al. (2010)	dHPC	2	Cannulae placement	Muscimol	20 min pre	+1 mm
Jo and Lee (2010)	HPC	2	Fluorescent injection	Muscimol	30 min pre	+1 mm
Kelemen and Fenton (2010)	Left or right HPC	2	N/A	TTX	1 h pre	+3 mm
Gomes et al. (2010)	CA1	2	Methylene blue injection	NMDAr antagonists	Post	+1 mm
Cohen et al. (2010)	dHPC	2	India ink injection	ZIP	1 h or 10 days post	+1 mm
Iordanova et al. (2009)	HPC	2	Cannulae placement	Muscimol	Immediate pre	+1 mm
Cimadevilla et al. (2009)	Unilateral HPC	1	Cannulae placement	Lidocaine or TTX	1 min post	+2 mm
McEown and Treit (2009)	dHPC or vHPC	2	Cannulae placement	Lidocaine	5 min pre or post training	N/A
Esclassan et al. (2009)	dHPC or vHPC	2	Cannulae placement	Muscimol	20 min pre	+1 mm
Czerniawski et al. (2009)	dHPC or vHPC	2	Cannulae placement	Muscimol	30 min pre	+1 mm
Klur et al. (2009)	Right and/or left HPC	2	Cannulae placement	Lidocaine	5 min pre	+1 mm
Tan (2008)	dHPC or vHPC	2	Cannulae placement	NMDAr antagonist	20 min pre	N/A
Atkins et al. (2008)	vHPC	2	Cannulae placement	Lidocaine	Just pre	N/A
Cimadevilla et al. (2008)	Unilateral or bilateral HPC	2	Cannulae placement	TTX	1 min post	+2 mm
Parsons and Otto (2008)	dHPC	2	Cannulae placement	Muscimol	30 min pre	N/A
Hafting et al. (2008)	HPC	2	Sub-population recording	Muscimol	Immediate	+0.9 mm
Atallah et al. (2008)	dHPC	2	Cannulae placement	Muscimol	15 min pre	+0.5 mm
Shahidi et al. (2008)	DG	2	Cannulae placement	Picrotoxin	5 min pre	+1 mm
Yoon et al. (2008)	dHPC	2	Cannulae placement	Muscimol	Pre	+1 mm
Chang et al. (2008)	dHPC	2	Cannulae placement	Lidocaine	5 min pre or immediate post	+1 mm
McHugh et al. (2008)	dHPC or vHPC	2	Cannulae placement	Muscimol	15 min pre	N/A
Howland et al. (2008)	dHPC or vHPC	2	Cannulae placement	Lidocaine	5–10 min pre	+1 mm
Cimadevilla and Axias (2008)	dHPC	2	Cannulae placement	TTX	30 min pre	+2 mm
Calfa et al. (2007)	dHPC or vHPC	4	Cannulae placement	Lidocaine	5 or 60 min post	+1–2 mm
Amaral et al. (2007)	HPC	2	Cannulae placement	Muscimol	Immediately post	+1 mm
Rogers and See (2007)	vHPC	2	Cannulae placement	Baclofen/muscimol	Immediately pre	N/A
Maren and Hobin (2007)	dHPC	2	Cannulae placement	Muscimol	20 min pre	N/A
Burman and Gewirtz (2007)	dHPC	N/A	Cannulae placement	NBQX and muscimol	Immediately post or 2 h post	+1 mm
Cimadevilla et al. (2007)	Unilateral HPC	2	Cannulae placement	TTX	15 min pre	+2 mm
Akbari et al. (2007)	DG	2	Cannulae placement	SB-334867-A	15 min pre	+0.5 mm
Stouffer and White (2007)	dHPC or vHPC	2	Cannulae placement	Muscimol	30 min pre	+0.5 mm
Bhatti et al. (2007)	HPC (mossy fibers)	2	Fast green dye infusion	Lidocaine	5–10 min pre	N/A
Bertoglio et al. (2006)	dHPC or vHPC	2	Evans blue infusion	Lidocaine	10 min pre or immediately post	+1.5 or +3 mm
Akbari et al. (2006)	CA1	2	Cannulae placement	SB-334867-A	15 min pre	+0.5 mm
de Lima et al. (2006)	dHPC	2	Methylene blue dye infused	Muscimol	Immediately post/1.5 or 24 h pre	N/A
Prado-Alcala et al. (2006)	HPC	2	Cannulae placement	TTX	Immediately post	N/A
Igaz et al. (2006)	HPC	2	Methylene blue dye infused	MEK1/2 inhibitor	Immediately post	N/A
White and Gaskin (2006)	dHPC	2	Methylene blue infused	Muscimol	30 min pre	+1 mm
Hobin et al. (2006)	vHPC	2	Cannulae placement	Muscimol	20 min pre	N/A
Gaskin and White (2006)	dHPC	2	Methylene blue dye infused	Muscimol	30 min pre	+1 mm