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Original Article

Histological Assessment of Thermal Damage in the Brain Following Infrared Neural Stimulation

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ABSTRACT

Background: Infrared neural stimulation (INS) is a novel technique for modulating neural function. Its advantages over electrical stimulation include high spatial specificity, lack of electrical artifact and contact-free stimulation. INS acts via a rapid, focal increase in temperature. However, in order to become a viable experimental and therapeutic tool, the safety of INS must be demonstrated.
Objective/hypothesis: Our aim was to determine the upper limit for the radiant exposure of INS in the brain without causing damage, using an INS sequence previously shown to induce both behavioral and electrophysiological effects in rodents and non-human primates.
Methods: We stimulated the brains of anesthetized rodents and two squirrel monkeys using an infrared laser, depositing radiant energies from 0.3 to 0.9 J/cm^2 per pulse in 0.5 s-long 200 Hz trains. At the end of the experiment, the animals were euthanized, perfused and the brains processed using standard histological techniques.
Results: Radiant exposures greater than or equal to 0.4 J/cm^2 resulted in identifiable lesions in brain sections. The lesions had a shape of a parabola and could further be subdivided into three concentric zones based on the type of damage observed.
Conclusions: The thermal damage threshold following our INS paradigm was between 0.3 and 0.4 J/cm^2 per pulse. This value is lower than the one found previously in peripheral nerve. The differences are likely due to the structure of the INS sequence itself, particularly the repetition rate. The results warrant further modeling and experimental work in order to delimit the INS parameter space that is both safe and effective.

Introduction

Within the past two decades, much progress has been made in the area of brain machine interfaces, with the goal of restoring function to impaired neural networks or otherwise altering or enhancing brain function. Central to these approaches has been the activation (or inactivation) of brain tissue via electrical stimulation. However, this conventional method suffers from lack of spatial precision due to current spread. Implantation of newer microelectrode arrays offers better current control but allows for only sparse coverage of the neuronal circuit due to the trauma caused by implantation of a dense wire array [1]. A fundamentally different approach is the use of light to stimulate neurons, a technique that overcomes the problem of current spread and offers focal and spatially specific neural stimulation. Recent advances capable of rendering neurons sensitive to light (by introducing algal photosensitive ion channels from the opsin protein family into the mammalian genome using recombination techniques or viral vectors), a method known as optogenetics, has revolutionized the field of neuroscience research [2]. Optogenetics allows one to focally stimulate specific neuronal populations (e.g. excitatory or inhibitory neurons). However, the prospect of using optogenetics as a clinical tool is still uncertain, due to concerns over the safety of gene therapy in human patients.

Another approach to using light to stimulate neural tissue is infrared neural stimulation (INS). This method employs brief pulses of infrared (1000–3000 nm) light to stimulate neurons [3]. Neuronal excitability by INS has been demonstrated in a variety of

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neuronal tissues, ranging from embryonic hearts to the human PNS and does not require chemical or genetic manipulations [8–11]. The transduction of optical pulses leads to changes in the transmembrane potential and is driven by the thermal gradient induced by the absorption of infrared light [4]. Recent *in vitro* studies using artificial lipid membranes and *Xenopus* oocytes have shown that the mechanism behind INS in these preparations is due to the temperature-dependent redistribution of capacitive charges across the lipid membrane itself [5]. The effect can be large enough to drive the membrane past the depolarization threshold. *In vivo*, in addition to the effects on the membrane itself, INS may also alter the conductance of various ion channels, many of which (the TRPV channel family, for example) are exquisitely temperature-sensitive [6]. The presence of these channels is one explanation for the observed differences to INS in terms of radiant exposure across various types of excitable membranes [7]. The spatial specificity of INS comes from: 1) the fact that its action is confined to the area being illuminated and 2) that lateral spread is minimal, due to low scattering, high absorption of mid-infrared light and the short duration of the light pulse (10^{-4}–10^{-3} s) compared to the thermal relaxation time of about 90 ms [4]. Around the 1900 nm absorption peak for water (other peaks exist around 1470 and 3000 nm), the absorption coefficient is 100 cm^{-1} and very focal activation can be achieved by delivering light to the region of interest via an optical fiber (e.g. 100–200 microns in diameter) [12].

Although INS has several advantages over electrical and optogenetic stimulation methods, its safety in the CNS needs to be fully assessed before it can be used in the clinic. The primary concern is thermally induced damage. Two questions need to be answered. First, what is the maximum temperature rise, T_{max}, tolerated by neural tissue? And, second, what is the parameter space for INS which produces temperatures below T_{max}? Neither of the two questions is easy to answer. The lower limit for T_{max} must lie somewhere around 41–42 °C, a point at which thermal damage to tissue has been observed in patients with high fevers and in animal studies [13]. Since INS is pulsed, and not continuous, this figure is probably too low. For example, human skin can be exposed for several minutes to immersion in water at temperatures of 48 °C, and can even tolerate temperature of up to 60 °C if the exposure is shortened to several seconds [14]. The upper limit is around 100 °C, the boiling point of water, at which immediate damage is observed. This range of 60 °C is too broad to be useful.

The temperature rise produced during INS is also not well established. It is difficult to measure empirically. Reported values differ considerably and have been obtained in different tissues, and, therefore, may not be comparable [4,15]. Moreover, the temperature during INS is dependent on a number of factors, including the repetition rate of the stimulation pulses and the energy delivered per pulse. A further complication arises from the fact that, at high pulse rates, the heat generated by each pulse does not dissipate completely and therefore the steady state temperature of the tissue increases. Given the rather large parameter space, the question is most easily addressed by numerical models, a number of which have been published [16–19]. However, these models often do not include a refined description of the tissue being stimulated, treating it as a homogenous aqueous environment.

Rather than trying to estimate T_{max} tolerated by the tissue and predict whether the particular INS paradigm used exceeds it, we took an empirical approach and looked for signs of tissue damage following INS, varying just one parameter, the radiant exposure per pulse. A previous study has examined the safety of applying INS to peripheral nerves in rats [20]. Our study differs significantly in that it employs both the INS sequence and its mode of delivery in a way that is directly applicable to neuroscience experiments in non-human primates, a primary model for human applications [21]. In this study, we obtain an approximate threshold above which thermal damage is observed in histological preparations for this particular INS sequence, characterize the changes in the severity of damage as a function of radiant exposure, and propose an underlying mechanism responsible for this damage.

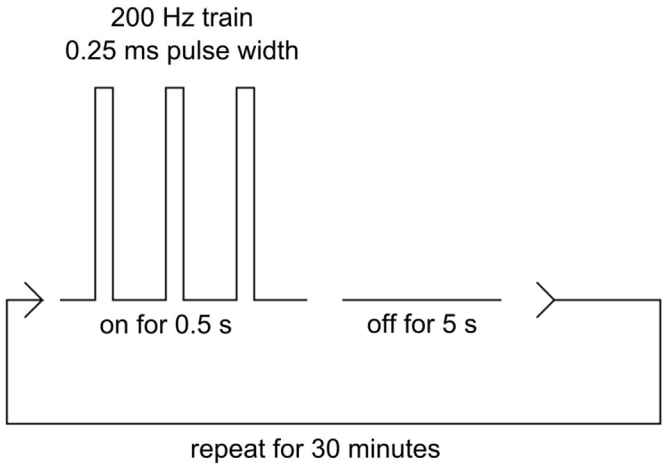

Figure 1. INS pulse sequence. We used the INS pulse sequence shown above throughout the study, with the exception of a few trials using single high energy pulses. It consisted of high-frequency trains of laser pulses expected to modulate brain function followed by periods when the laser was off and the cortex was allowed to recover. To simplify interpretation of results, we kept the timing of the sequence constant and only varied the power (by adjusting the laser diode current) to alter the dose of radiant energy delivered to the cortex.

Methods

Stimulation sequence

The INS stimulation paradigm that we tested was developed in view of its potential application, namely, modulation of cortical activity in awake, behaving non-human primates. It is structured as a series of short INS bursts with a longer interstimulus interval between, corresponding to a number of trials back-to-back within one experimental session (Fig. 1). The bursts are 0.5 s in duration and consist of a continuous train of 0.25 ms wide pulses delivered at a frequency of 200 Hz. These pulse trains are sufficient for eliciting neuronal activation observed using calcium reporter dyes and electrophysiological and optical imaging of hemodynamic response measurements in rodents [10]. The only variable in the stimulation sequence was the radiant exposure per pulse. Since the pulse duration remained constant, this was achieved by altering the laser diode current and thus peak powers were adjusted to deposit from 0.3 to 0.9 J/cm^2 for each pulse. The inter-stimulus interval between the 0.5 s pulse trains was 5 s, and was chosen because it was deemed long enough to allow for the residual heat to dissipate, yet short enough to allow for a large number of stimulations to be performed within a reasonable amount of time. The stimulation sequence was run for 30 min, resulting in approximately 36,000 individual pulses being delivered to the brain. Since the damage threshold for the stimulation sequence was not known in advance, we used high-energy single pulses (2 or 5 J/cm^2 delivered in 2 or 5 ms, respectively) to estimate the upper limit for the energies required. Single pulses of either magnitude produced large lesions; therefore we decreased the diode current by a factor of two or more for the pulse sequence.