Partial antagonism of propofol anaesthesia by physostigmine in rats is associated with potentiation of fast (80–200 Hz) oscillations in the thalamus

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Editor’s key points

- The thalamus is involved in anaesthesia.
- In this study, propofol anaesthesia in rats was antagonized by physostigmine.
- Increased thalamic activity was seen when physostigmine was given during propofol anaesthesia.
- Impaired thalamic function is associated with anaesthesia-induced unconsciousness.

Background. Positron emission tomography studies in human subjects show that propofol-induced unconsciousness in humans is associated with a reduction in thalamic blood flow, suggesting that anaesthesia is associated with impairment of thalamic function. A recent study showed that antagonism of propofol-induced unconsciousness by the anticholinesterase physostigmine is associated with a marked increase in thalamic blood flow, supporting the implication of the thalamus. The aim of the present study was to assess the role of the thalamus in the antagonistic effects of physostigmine during propofol anaesthesia using electrophysiological recordings in a rat model.

Methods. Local field potentials were recorded from the barrel cortex and ventroposteromedial thalamic nucleus in 10 chronically instrumented rats to measure spectral power in the gamma/high-gamma range (50–200 Hz). Propofol was given i.v. by target-controlled infusion at the lowest concentration required to abolish righting attempts. Physostigmine was given during anaesthesia to produce behavioural arousal without changing anaesthetic concentration.

Results. Compared with baseline, gamma/high-gamma power during anaesthesia was reduced by 31% in the cortex (P=0.006) and by 65% in the thalamus (P=0.006). Physostigmine given during anaesthesia increased gamma/high-gamma power in the thalamus by 60% (P=0.048) and caused behavioural arousal that correlated (P=0.0087) with the increase in power. Physostigmine caused no significant power change in the cortex.

Conclusions. We conclude that partial antagonism of propofol anaesthesia by physostigmine is associated with an increase in thalamic activity reflected in gamma/high-gamma (50–200 Hz) power. These findings are consistent with the view that anaesthetic-induced unconsciousness is associated with impairment of thalamic function.

Keywords: acetylcholine; anaesthesia; anticholinesterase; barrel cortex; electrophysiology; general anaesthetics; local field potentials; rat; thalamus

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The mechanisms by which general anaesthetics cause unconsciousness are not fully understood.1,2 Positron emission tomography studies have shown that unconsciousness induced by propofol is associated with reductions in thalamic blood flow,3,4 suggesting that the thalamus has a role in how anaesthetics impair consciousness.1,2 A recent study has shown that antagonism of target-controlled infusion (TCI) of propofol anaesthesia by the anticholinesterase physostigmine in human subjects is associated with a marked increase in thalamic blood flow, further supporting the implication of the thalamus in the changes in the level of consciousness.5 The aim of the present study was to use electrophysiological recordings in an animal model to assess the role of the thalamus in the antagonistic effects of physostigmine during propofol anaesthesia. Blood flow measures provide an indirect measure of neuronal activity, and assume intact coupling between metabolism and blood flow.6 Electrophysiological recordings offer complementary evidence of changes in thalamic activity by providing a direct measure of neuronal activity.6

We have found previously in unpublished studies that physostigmine also antagonizes propofol anaesthesia in rats as revealed by spontaneous movements of the limbs and of the head, by spontaneous whisker movements, and by orientating with occasional biting after gentle touch of the snout with a swab. We now report the results of
recording thalamic and cortical local field potentials (LFPs) to measure the spectral power of spontaneous oscillations in the gamma (51–80 Hz) and high-gamma ranges (81–200 Hz) during antagonism of TCI propofol anaesthesia by physostigmine in rats. These frequency ranges were chosen because these oscillations correlate tightly with changes in regional cerebral blood flow and provide a very useful index of brain activation. Furthermore, intracranial cortical recordings from human patients show that propofol attenuates power in the 50–200 Hz range, and intracranial recordings from rats also show that isoflurane anaesthesia reduces power in the 70–140 Hz range. We did not include the low gamma (30–50 Hz) range, which has limited usefulness to assess anaesthetic effects. We predicted that the spectral power in the 50–200 Hz range would be reduced during anaesthesia with propofol for both the thalamus and the cortex, and that the arousal response induced by physostigmine would be accompanied by an increase in power in the thalamus.

Methods
All procedures adhered to the guidelines of the Canadian Council on Animal Care, and were approved by the Animal Ethics Boards of Concordia and McGill University. Male Long–Evans rats (300–350 g, n=14) were acquired from Charles River Laboratories (Senneville, Quebec, Canada). They were housed individually, provided food and water ad libitum, and maintained on a 12 h reversed light cycle (lights on from 20:00 to 08:00 h).

Surgery
Recording microelectrodes were implanted stereotaxically under isoflurane anaesthesia in the ventroposteromedial nucleus (VPM; stereotaxic coordinates: P, −3.4; L, 2.5; V, 6.4 mm relative to bregma) and in the barrel cortex (P, −2.0; L, 4.6; V, 2.0 mm). A catheter suitable for long-term use was also inserted in the right jugular vein. Full details of the procedures are described in the Supplementary material. After surgery, catheters were flushed daily with a dilute solution of gentamycin and heparin and 10 days were allowed for recovery. After completing the testing sessions, the animals were killed with a lethal dose of urethane and perfused via the left ventricle with heparinized saline followed by 10% neutral buffered formalin. The isolated brains were post-fixed in 4% paraformaldehyde for histological processing and confirmation of correct placement of the electrodes.

Design
There were two testing sessions, separated by at least 3 days. In the first session, physostigmine was injected as a reversal agent during anaesthesia. In the second session, normal saline was injected as a control reversal agent. Physostigmine was tested first to maximize the number of observations for the active drug in the event that an animal would not complete the entire experimental sequence. Each session consisted of four periods: baseline, anaesthesia, attempted reversal of anaesthesia with either physostigmine or a saline injection, and recovery. Five of the 10 animals had been previously exposed to physostigmine as part of a related study assessing the effects of physostigmine during inhaled isoflurane anaesthesia. Results from naïve rats were similar to those from rats that had been previously exposed to physostigmine, and data from both groups were therefore pooled. The results of the study with isoflurane will be reported separately.

After baseline recordings of LFPs, the propofol tubing was connected to the jugular vein catheter via one arm of a Y-connector (Interlink System, Baxter Healthcare Corporation, Deerfield, IL, USA). The infusion rate of propofol was adjusted by a Harvard 22 syringe pump controlled by the Stanpump software developed by Steven L. Shafer and colleagues (Department of Anesthesiology, Stanford University, CA, USA) using pharmacokinetic parameters derived by Knibbe and colleagues. The initial target plasma concentration was set at 4 μg ml−1. It was increased by 0.5 μg ml−1 every 2 min until the animal made no attempts to right itself when placed sequentially on its right and left sides. The range of final target plasma concentrations was 7.0–10.0 μg ml−1. With this level of anaesthesia, the animals made no spontaneous movements and did not react when their snout was gently touched. Recording of the LFPs during anaesthesia was initiated 6–8 min after reaching the final target plasma concentration. After these recordings, physostigmine (0.4 mg kg−1) mixed with glycopyrrolate (0.08 mg kg−1), or an equivalent volume of normal saline, was injected over 2 min via the other arm of the Y-connector. This dose was chosen because behavioural pilot tests with physostigmine had revealed a ceiling effect for doses of 0.30 mg kg−1. Glycopyrrolate, a muscarinic blocker that does not cross the blood–brain barrier, was always given with physostigmine to prevent the peripheral muscarinic side-effects. The injection of physostigmine occurred [mean (SD)] 29.4 (5.4) min after reaching the final target plasma concentration. That of saline occurred 36.4 (6.4) min after reaching the final target plasma concentration. After this injection, the animals were observed for signs of arousal, and the following behaviours were immediately recorded on an itemized scoring sheet as absent, mild, or moderate in intensity (0, 1, or 2, respectively): spontaneous movements of the limbs, of the head, of the whiskers, and orientating after gentle touch of the snout with a swab. Overall arousal was ranked based on the following sum: orientating score + whisker movements score + head movements score + [limb movements score]/2. Reduced weight was given to the limb movement score as it sometimes included fasiculations, which could reflect an isolated muscle response to the anticholinesterase. The righting reflex was assessed, and LFPs were recorded 2–3 min after the appearance of the arousal response, or 10 min after the injection if the animal showed no signs of arousal. A final set of LFPs recordings was obtained during recovery from anaesthesia 10 min after the return of ambulation. Rectal temperature was maintained at 36.5–37.0°C with heat pads placed beneath the chamber.
Electrophysiological recordings

Both referential and bipolar recordings were obtained. We only report the results of the bipolar recordings because referential recordings are more subject to contamination from volume-conducted myogenic artifacts. LFPs from the cortex and thalamus were amplified (0.1–500 Hz pass band; A-M Systems, Model 1700), digitized at 1024 Hz and stored for offline analysis. For each period, 2 min of high quality data devoid of artifacts was obtained.

Signal processing

Spectral power was computed with Welch’s method using 2 s long non-overlapping segments and a Hamming window (Matlab Signal Processing toolbox, version 6; MathWorks Inc., Sherborn, MA, USA). Segments containing more than 5% outliers [defined as values outside mean (3·SD) of the entire recording of ~2 min] were excluded from analysis. Frequency resolution was 0.5 Hz. We chose Welch’s method of spectral analysis because it is one of the most popular in the EEG literature, including Hudetz and colleagues. The use of the more complex multitapering approach to spectral analysis may offer better protection against 60 Hz contamination, but we found after analysis of a partial sample of data that both methods yielded equivalent results. Details of how the impact of 60 Hz noise was minimized are given in the Supplementary material.

Statistics

Results for the gamma (51–80 Hz) and high-gamma (81–200 Hz) bands were similar, and data for the two bands were therefore pooled. For each recording site, the differences in spectral power between successive periods (baseline compared with anaesthesia, anaesthesia compared with physostigmine, and physostigmine compared with recovery) for the pooled gamma/high-gamma frequency range (51–200 Hz) were compared with the Wilcoxon matched paired test. The P-values of each set of three comparisons were adjusted for multiple comparisons with Hommel’s procedure. To measure the association between the intensity of behavioural arousal and changes in spectral power, we used the Spearman rank-order correlation coefficient. The criterion for statistical significance was P ≤ 0.05. Hommel’s procedure was computed with SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) and all other procedures were implemented in Matlab version R2007b (MathWorks, Natick, MA, USA). All logarithmic transformations in the figures are in base 10.

Results

The results are based on a group of 10 animals that completed both the physostigmine and saline sessions.

Physostigmine sessions

Cortical gamma/high-gamma power decreased significantly during anaesthesia (P = 0.006) and there was a trend towards a further decrease after physostigmine (P = 0.065) (Fig. 1). Recovery was associated with a significant increase in cortical power (P = 0.01). Thalamic gamma/high-gamma power also decreased significantly with anaesthesia (P = 0.006) and increased significantly after the injection of physostigmine (P = 0.048). Recovery was associated with a significant increase in thalamic power (P = 0.027). Therefore, physostigmine causes a significant increase in fast activity only in the thalamus.

Normal saline sessions

Cortical gamma/high-gamma power decreased significantly during anaesthesia (P = 0.008) and was further decreased after normal saline (P = 0.01) (Fig. 1). Recovery was associated with a significant increase (P = 0.006) in power compared with the saline period. Similarly, thalamic gamma/high-gamma power decreased significantly with anaesthesia (P = 0.014), decreased further after injection of saline (P = 0.004), and increased during recovery (P = 0.004). We were puzzled by this unanticipated decrease in power in both the cortex and the thalamus after saline. The search for an explanation led us to realize that the administration of saline (and physostigmine) was associated with the unintentional administration of a small bolus of propofol present in the shared catheter tubing (volume of 0.1 ml) below the Y-connector linking lines containing propofol and saline. This issue is addressed in Supplementary Figure S1.

Behavioural effects of physostigmine during anaesthesia and electrophysiological correlates

Although no animals regained the righting reflex, the administration of physostigmine during anaesthesia resulted in clear signs of arousal (Table 1). Mild-to-moderate limb movements were almost always present and other signs of arousal were frequently observed. The intensity of behavioural arousal induced by physostigmine during propofol anaesthesia correlated tightly with an increase in thalamic power in the gamma/high-gamma (51–200 Hz) range (Spearman’s coefficient 0.773; P = 0.0087) (Fig. 2a). The intensity of the arousal was also inversely related to the target concentration of propofol (Spearman’s correlation coefficient = 0.72, P < 0.02, Table 1).

The power spectra from two animals that showed a high and low arousal response to physostigmine (animals #2 and #4, respectively) are shown in Figure 2b. The effect of physostigmine on the thalamic recording of rat #2 who showed strong behavioural arousal is readily visible (filled arrow), whereas minimal changes were observed for rat #4 (open arrow). Figure 2c shows representative raw tracings from the same animals. Physostigmine caused noticeable activation of the LFPs (decrease in low-frequency activity with an increase in high frequency) in rat #2 (filled arrows) but minimal, if any, changes in animal #4 (open arrows).

Averaged spectra

The average spectra for all animals combined are shown in Figure 3. The changes revealed by these spectra match those described in Figure 1. The average spectra show that
gamma/high-gamma power decreased during anaesthesia compared with baseline for both sessions (physostigmine or saline) and recording sites (cortex and thalamus). They also reveal an unequivocal increase in thalamic gamma/high-gamma power after physostigmine. In contrast, the injection of saline was associated with a clear decrease in thalamic gamma/high-gamma power. Cortical gamma/high-gamma power also decreased after saline.

Table 1  Behavioural response after physostigmine during propofol anaesthesia. ID, rat number; Propofol conc., target plasma propofol concentration in $\mu$g ml$^{-1}$; LM/2, limb movement score divided by 2; HM, head movements. Number denotes intensity of movements: 0, absent; 1, mild; 2, moderate

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Fig 1  Power in the 50–200 Hz range in the barrel cortex and ventroposteromedial thalamus for tests that used either the anticholinesterase physostigmine (left) and/or saline (right) injections during propofol anaesthesia. A thin line indicates data for each animal, and a thick line shows the median power. Power was calculated during the baseline period (base), propofol anaesthesia (propo), after injection of either physostigmine (physo) or saline, and after recovery (reco) from anaesthesia. P-values for pair-wise comparisons are also indicated (Wilcoxon matched pairs test; corrected for multiple comparisons).
Histology
Correct placement of the electrodes was confirmed in nine of the 10 brains (Supplementary Fig. S2) of the first group of animals. The other brain was damaged during processing and the slices could not be interpreted. No histology was performed in the four animals where revised administration of physostigmine was undertaken.

Discussion
Propofol anaesthesia was associated with a large and significant decrease in gamma/high-gamma power in both the barrel cortex and VPM thalamus. The administration of physostigmine during propofol anaesthesia was followed by behavioural arousal consistent with partial antagonism of propofol action, and also an increase in gamma/high-gamma power in the VPM. Furthermore, this increase in power was tightly correlated with the intensity of behavioural arousal. These findings support previous observations showing that the antagonism of propofol anaesthesia by physostigmine is associated with thalamic activation in humans. They are thus in line with the view that alteration of thalamic function is one of the key factors mediating the hypnotic action of general anaesthetics.

We chose the ventroposteromedial thalamic nucleus (VPM) because of its documented sensitivity to general anaesthetics and because it is suitable for recording spontaneous fast oscillations. We chose the barrel cortex because it receives inputs from the VPM and also displays fast spontaneous oscillations. We did not expect a major effect of physostigmine on the barrel cortex because physostigmine during propofol anaesthesia in humans did not increase blood flow in the primary somatosensory cortex. The main increase in cortical blood flow in that study occurred in the cuneus and precuneus, areas that have no equivalent in the rat.

Physostigmine antagonized anaesthesia in the present study more weakly than has been observed previously in...
There was an inverse relationship between the intensity of arousal and anaesthetic concentration in the present study, suggesting that the ability of physostigmine to antagonize propofol decreases at higher propofol concentrations. This probably also occurs with human subjects. This could explain why physostigmine is more effective at reversing propofol anaesthesia in humans than in rats, because rats require a concentration of propofol three times higher than humans for the induction of unconsciousness.

The administration of saline was followed by a small but significant, unexpected decrease in gamma/high-gamma power in both the cortex and VPM (Figs 1 and 3). We initially attributed this decrease to a deepening of anaesthesia caused by the unintentional administration of a small (0.1 ml–1 mg) bolus of propofol along with the saline. However, the increase in propofol concentration caused by the bolus would likely be offset by the presence of saline in the tubing. With the injection of saline, the animals initially received more propofol than that determined by the TCI system. But immediately afterwards, the rats received less propofol than that calculated by the TCI system because propofol in the line was diluted by saline. Since the infusion rate was $\approx 0.05 \text{ ml min}^{-1}$ and the shared tubing volume 0.1 ml, the effect of the injecting saline in the tubing would be negligible after a few minutes. Other explanations for the gamma/high-gamma power decrease must thus be considered. These include inaccuracy of the TCI model (i.e. a progressive increase in the propofol concentration with time) or increased sensitivity to propofol with time. Exploration of these issues is beyond the scope of the present study.

Although the unintentional administration of a small (0.1 ml–1 mg) bolus of propofol likely did not cause a sustained increase in the plasma concentration of propofol, there remained the possibility that the behavioural and electrophysiological responses to physostigmine may have been blunted by the concurrent administration of a bolus of propofol. However, a follow-up experiment using injections designed to minimize the entrainment of propofol during injection of physostigmine (see Supplementary Material) revealed effects on behaviour and thalamic gamma/high-gamma power similar to those observed with the first groups of rats. We thus conclude that the small propofol bolus that may have accompanied the injection of physostigmine in the first group of rats did not change the response to physostigmine on behaviour and thalamic power.

The effects of physostigmine likely follow from increased muscarinic tone caused by higher acetylcholine availability. Muscarinic receptor activation potentiates fast

![Fig 3](average_power_spectra_over_the_gamma/high-gamma_range_50-200_Hz_for_all_animals_combined_for_both_sessions_physostigmine_and_saline_and_recording_sites_Cx_cortex_Th_thalamus_mean_sem_shown_for_every_12th_data_point_for_clarity_blank_areas_at_60_120_and_180_Hz_show_the_power_ranges_excluded_from_analysis_to_avoid_contamination_from_power_line_noise_in_comparison_with_baseline_gamma/high-gamma_power_decreased_during_anaesthesia_thalamic_gamma/high-gamma_power_increased_after_physostigmine_and_decreased_after_saline_cortical_gamma/high-gamma_power_also_decreased_after_saline)
oscillations\textsuperscript{24, 25} and propofol inhibits M\textsubscript{1} receptor-mediated signal transduction.\textsuperscript{26} Physostigmine may therefore potentiate fast neuronal oscillations by increasing acetylcholine availability to antagonize the effects of propofol on M\textsubscript{1} receptors. However, the effects of physostigmine may also be mediated in part by nicotinic effects, because nicotinic activation can also enhance gamma oscillations.\textsuperscript{27} Although the precise mechanisms for the effect of physostigmine need to be determined, we conclude that partial antagonism of propofol anaesthesia by physostigmine is associated with an increase in thalamic activity measured with gamma/high-gamma (50–200 Hz) power. These findings are consistent with the view that anaesthetic-induced unconsciousness is associated with impairment of thalamic function.\textsuperscript{1, 2}

**Supplementary material**

Supplementary material is available at *British Journal of Anaesthesia* online.

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**Declaration of interest**

None declared.

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