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The effects of halothane gas anesthesia on the visual neuronal activities in the feline caudate nucleus

Summary of PhD Thesis

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Introduction

The primary input structure of the basal ganglia, the caudate nucleus (CN) receives projections from the whole cerebral cortex, therefore it is involved not only in motor but also in various higher neurological functions. The CN contains neurons, which are sensitive to different modalities of visual stimulation. The visual receptive field and the response characteristics of these neurons makes them to be markedly sensitive to dynamic stimuli, therefore it is assumingly involved in perception of self-motion in the visual environment. The striatal neurons can be classified in three main neuronal groups according to their electrophysiological properties: phasically active neurons (PANs), tonically active neurons (TANs) and high firing neurons (HFN). Most of the striatal neurons are the medium spiny neurons (MSN) which are the main contributors of the cortico-striatal and thalamocortical loops and which most probably corresponds to the PANs. The parvalbumin-expressing GABAergic interneurons can be characterized as HFNs based on their electrophysiological properties. These neurons have relatively high spontaneous firing rates between 10-30 Hz in behaving animals. The other major interneuron type in the striatum contains the cholinergic interneurons, which seems to overlap with the TANs.

The visually evoked low-frequency signals (local field potentials (LFPs)) changes can carry information about the cortical and subcortical visual inputs in the striatum. Low-frequency LFP oscillations can coordinate communication among brain regions. Theta-band (6-10 Hz) LFP rhythms occur in the dorsolateral striatum of rodents, and it is associated with the modulation of behavior related information processes between the striatum and cortex or hippocampi. Increased theta band of the LFPs could be associated with the maintenance and the encoding of visual information in visually-guided short-term memory. Alpha-band LFP (8-13 Hz) rhythms seem to be associated with visual perception, such as target detection and could be involved in temporal resolution of perception. Beta-band oscillations (15-30 Hz) can be involved in the modulation of decision-making in the sensorimotor network, by encoding categorical choices. Normal beta band activity is also presumed to be essential cortical outcome of the normal basal ganglia function during visual information processing. Gamma oscillations (above 30 Hz) could also be play role in cognitive processes, such as word learning, reading, memory processes, behavior related modulation (i.e. reward approach and receipt and reward anticipation).

Earlier studies denoted that anesthetics have only minor influence on the visual responsiveness and the background activity in several brain structures. However, the effects of anesthesia do not necessarily have the same effects in the different brain structures. The basal ganglia were proved to be extremely sensitive to anesthesia. In the striatum different anesthetics have different effects on neuronal activity. Based on our previous experiences, halothane had least influence on the neuronal activities in the CN compared to other gas anesthetics. In order to eliminate the confounding effects of anesthesia, it was necessary to turn towards the use of the awake, behaving animal models in electrophysiological experiences. However, this raises many problems in visual electrophysiology because the control for eye movements is inevitable. One possible solution to stabilize eye movements is to use anesthetics with muscle paralysis on animals. However, anesthesia can also influence neuronal activity and a long-term data collection is not possible, which could increase the number of animals sacrificed in these experiences. Due to these disadvantages, awake, behaving animals are often used in visual electrophysiological research. However, these experiments are preceded by a long-lasting training period in which the animal have to be trained to maintain fixation in order to eliminate or at least reduce the effects of eye movements on the neuronal activities. Several years ago, our research group introduced an awake, behaving feline model that is suitable for visual electrophysiological recordings. The advantages of this model are the extended recording time of up to 1-2 hours a day for years and the continuous eye movement control, with eye tracking. Although this model has many advantages, the preparation of the animals is a challenging process and may take up to long (6-12) months.

Aims of the study

Based on these described earlier the question arises whether the benefit of experiments performed in the CN of awake, behaving animals outweighs the hardness of the preparation of the animals for visual electrophysiological experiments. In order to address this question, single-cell activity and LFP were recorded in the CN during visual stimulation (static and dynamic stimulation) from anesthetized, paralyzed and awake, behaving cats. The activity of CN neurons and their visual responses as well as the LFPs and visually evoked LFP changes were analyzed and compared in the two (anesthetized and behaving) feline models.

Materials and methods

Acute (anesthetized) experiments

Two versions of anesthetized experiments were performed. In the first anesthetized cat experiments (A experiment) the extracellular recordings were carried out with tungsten microelectrodes (20 kHz sampling rate) whereas in our next anesthetized experiments (B experiment) the LFP recordings were performed with 64-channel 2 shank (32 channel/shank, diameter: 300 μm/shank) platinum-iridium linear probes (20 - 28 kHz sampling rate). The recordings took place in a dark and quit room. Halothane anesthesia was applied to 0.8-1.0% during recordings to minimalize the effects of anesthesia on the neuronal acitvities. The visual stimulation was generated by custom made script in Matlab using the Psycho Toolbox in the A experiment and a custom-made software in the B experiment. The visual stimulation consisted of three steps: first a blank, black screen was presented to record background activity without any visual stimulation (intertrial interval: 1500 ms in the A and 2000 ms in the B experiments). In the following random dot patterns appeared (static visual stimulus: 1500 ms) and the same dots were moved either toward the periphery or the center of the screen (dynamic stimulus, center-in and center-out flow field: 1500 ms). The stimuli with center-in or center-out were presented in a random order.

Chronic (behaving) experiments

In these experiments, the recordings were preceded with the behavioral training of the cats. They were adapted to the laboratory environments and the to the suspension in a canvas harness, which leaves the head, legs and tail free. At first, they were lifted manually only a few centimeters from the floor in the canvas harness, then they were introduced to the experimental stand. After the cats accustomed to this circumstances the cats underwent surgery where scleral search coil was implanted into one eye in order to monitor the eye movements of the cats during the recordings and a stainless steel headholder was implanted on the skull to fixate the head in the recording sessions. Craniotomy was performed, where eight wire electrodes covered by a guiding tube were implanted above the CN. After the full recovery of the cats, the behavioral training continued. The head of the cats was fixed to the stereotaxic frame by the implanted steel headholder. The stereotaxic frame was placed within an electromagnetic field, which is generated by metal coils, installed into the wall of the experimental stand. The animals were then trained to hold their eyes

in the center of the monitor during recordings of neuronal activities to different kind of visual stimulation. The initial size of the fixation acceptance window was 10º around the center of monitor, which was reduced to 5º during the training. If the cats could hold the fixation during the whole trial of the task the animals received pulpy food as reward. The visual stimulation was generated by a custom-made script written in Matlab and using the Psychtoolbox ®. The visual paradigm (one single trial) consisted of 5 steps. A green fixation point was projected on the center of the monitor, where the cats had to direct their gaze to the fixation point and had to keep fixation for 500 ms. The static visual stimulation (random dot pattern) appeared on screen after the successful fixation phase for 1000 ms. After it the dynamic stimulus arrived (center-in or center-out optic flow, respectively) for 1000 ms. If the cat could hold the fixation during the whole visual stimulation protocol it was reward with pulpy food. After the rewarding phase the intertrial interval arrived without any stimulation, where the black screen was presented for 5000-10000 ms to record background activities. The extracellular electrophysiological recordings were carried out with parylene isolated platinum-iridium wire-electrodes for the first cat; and a formvar insulated nickel-chrome wire-electrodes for the second cat (10 kHz sampling rate).

Data analysis

For the single cell activity analysis, the raw data was processed by Neuroscope, NDManager, KlustKwik softwares: the amplified neuronal activities were band-pass filtered between 300-3000 Hz and after the threshold calculation the spikes were extracted based on the principal component analysis. This was followed by the manual spike sorting in the Klusters software, by testing the autocorrelograms and overlaid spike shapes. The classification of the neurons were maintained by using the background discharge rate, propISI>2sec and the shape of the two autocorrelograms (±100 ms and ±1000 ms). Based on these parameters, the striatal neurons could be separated into three main electrophysiological categories: phasically active neurons (PANs), high firing neurons (HFNs) and tonically active neurons (TANs). Peristimulus time histograms (PSTH) were made from the temporal distribution of the spikes for each single neuron. Evoked activities during the static and dynamic stimulation and the background activities were compared (Wilcoxon matched pair test). If the firing rates were significantly different ($p<0.05$) from the background activity, we considered the neuronal activity as a response. The striatal neurons with background activities under 1 spike/sec were excluded from further analysis. Both the gross and net activities were

calculated, to gain information about the overall neuronal activity and to get the magnitude of visually evoked responses. Mann-Whitney test was used to compare the background activities from the anesthetized and behaving cats at the population level.

Matlab R2021a software was used for data analysis and statistical analysis of the power of the delta (1-3 Hz), theta (4–7 Hz), alpha (8–13 Hz), beta (14–30 Hz), and gamma (31–70 Hz) frequency bands of the LFPs. The raw data were downsampled to 500 Hz. This was followed by the Fourier analysis, then the power spectrum was video-filtered by a ten-bin rectangle window. Bins around 50 Hz were cut off . For the purpose of reaching the desired width of the band the filtering (finite impulse response filter (FIR) decimation filter, length 20 ms) and decimating (Chebyshev infinite impulse filter (IIR)) steps were repeated, until we reached 25 Hz sampling frequency, which is also suitable for the analysis of lowest (even interesting) theta band. The higher frequency bands were then calculated and decimated with the same time resolution and sampling frequency. 320 ms periods were randomly selected from the background activity and from the start of the stationary and dynamic stimulus from both the behaving and anesthetized LFP recordings. The powers of these periods in each frequency band from all trials were compared with Friedmann ANOVA test with Bonferroni correction. Significant difference ($p<0.05$) in the LFP recording means that at least one of the three conditions (background activity, response to static or dynamic stimulation) differs from one another. In order to check exactly which part of the stimulated activity (static, dynamic, or both) differs significantly from the background activity Wilcoxon matched pair test was used.

Results

From the A anesthetized experiments altogether 206 neurons were recorded in the CN, whereas 344 neurons were recorded in the CN from the two awake, behaving cats.

The background activity of CN neurons was significantly higher (Mann–Whitney U, $p < 0.001$) in awake, behaving cats compared to that of CN neurons recorded in anesthetized cats. Due to the high prevalence of CN neurons exhibiting extremely low background activity, with a separate analysis those neurons which background activity were below 1 spike/s were excluded. This was necessary to exclude potential biases in the analysis. This resulted the exclusion of 135 neurons from the awake (39.2%) and 125 neurons from the anesthetized model (60.7%). A comparison of

background activities exceeding 1 Hz demonstrated a significantly higher activity of CN neurons recorded from the behaving cats (Mann-Whitney U, $p < 0.001$).

The stimulated activities (static and dynamic stimulus) were compared to the background activities. The proportion of neurons, which showed responsiveness to static stimulation was greater in behaving cats. Both gross and net activity increments during static stimulation were notably stronger in the behaving cats compared to the anesthetized animals. Additionally, the magnitude of net activity decrements during static visual stimulation was more apparent in the behaving model. Similarly, to the responsiveness to static stimulation, the proportion of neurons responsive to dynamic stimulation was higher in the behaving cats. During dynamic stimulation the increased activities were notable in behaving cats, however, no such responses were seen in the anesthetized animals at all.

The vast majority of the registered CN neurons in the awake, behaving cats were PANs (N = 219, 63.7%) but less number of HFNs (N=88, 25.6%) and TANs (N=28, 8.1%) were recoreded. On the contrary, in the anesthetized cats the majority of the recorded neurons were HFNs (N=85, 41.3%) with fewer PANs (N=73, 35.4%) and even fewer TANs (N=43, 20.9%). In behaving cats, the background activities in each electrophysiological group were significantly higher compared to those of the anesthetized model. Due to their low background activity, most of the neurons excluded from the analysis were PANs in both feline models. However, due to the applied 1 Hz cut-off criteria resulted in the exclusion of all PANs in the anesthetized animals. The background activities of both HFNs and TAN groups were significantly higher in the awake animals.

In the data analysis of LFPs a total of 226 LFP recordings were included from the awake, behaving cats and 960 LFPs from the anesthetized cats recorded in the B anesthetized experiments.

All LFP recordings were analyzed in five different frequency bands, i.e. delta (1-3 Hz), theta (4-7 Hz), alpha (8-13 Hz), beta (14-30 Hz) and gamma frequency bands (31-70 Hz). Comparison of the powers revealed (Wilcoxon matched pair test) that 90% (N=207) of all registered LFPs from awake cats exhibited significant responses in at least one visual condition (static, dynamic) or at least one frequency band. This was notably less in the anesthetized cats, where only 63% (N=609) of the LFPs showed significant responses to visual stimulation.

In the awake, behaving animals the percentage of visually evoked LFPs in the theta band was 43% (N=98), in alpha was 56 % (N=121) and 64 % (N=114) in the beta frequency band. On the contrary, in the anesthetized animals the percentages were lower, in the theta band it was only 19% (N=178),

19% (N=181) in the alpha band and 22% (N=210) in the beta frequency band. The percentage of LFPs with significant changes showed no difference in the gamma frequency band between the two models, it was consistent at 23% for both awake and anesthetized cats (N=34).

The percentage of LFPs exhibiting significant response during static visual stimulation was higher in awake, behaving cats (83%, N=187) compared to that of the anesthetized cats (63%, N=602). Visually evoked LFP changes were observed at a higher percentage in nearly every frequency range in the behaving animals. The most notable differences were observed in the alpha and beta frequency bands. For the alpha band, the percentage was 49% (N=110) in awake cats, contrasting with only 24% (N=228) in anesthetized cats. Similarly, in the beta band, the percentage of significant LFP changes was 49% (N=110) for behaving cats, in contrast to 23% (N=221) for anesthetized cats.

In case of dynamic stimulation, in the behaving model a slightly lower proportion of LFPs (76%, N=172) showed significant responsiveness compared to the impact of static stimulation, where the proportion was 83% (N=187). However, the percentages of LFPs responsive to static and dynamic stimulation were comparable in anesthetized animals, measuring at 63% (N=602) and 64% (N=611), respectively. The differences in the proportion of significant visually evoked LFPs were more prominent in the case of theta, alpha and beta frequency bands. In the theta band, the percentage was 39% (N=89) in awake cats and only 19% (N=183) in anesthetized cats. In the alpha band, the percentage was 34% (N=76) in behaving animals whereas it was 15% (N=147) in anesthetized cats. In the beta frequency band, the percentage of significant LFP changes was 31% (N=69) in behaving cats, whereas it was lower at 21% (N=37) in anesthetized cats.

Discussion

In visual electrophysiological experiments both anesthetized, paralyzed and awake, behaving animal models are widely used. The goal of this PhD thesis work was to give answer to the fundamental question whether neuronal activities to visual stimulation in the mammalian neostriatum in both single-cell and local field potential (LFP) level are comparable in these two models or no. The justification of this question is that the use of behaving animal model is more difficult than the anesthetized model. This raises the question, whether the costs and benefits are in equilibrium or no.

Concerning the background discharge rates of the recorded CN neurons, the majority of the units had less than 1 Hz activity in the anesthetized cats, thus these CN neurons had to be excluded from further analysis. Much less neurons had to be excluded for this reason in case the awake model. Additionally, the visual responsiveness to static and dynamic visual stimulation of CN neurons was significantly reduced in the anesthetized animals. The increased neuronal discharge rate was obvious in the awake cats during visual stimulation, however no such clear responses were seen in the anesthetized animals.

Based on the electrophysiological properties, the recorded CN neurons were separated into three different classes: phasically active (PANs), high firing (HFNs), and tonically active (TANs) neurons. In case of the awake feline model, our results are in line with earlier studies: the vast majority of the recorded CN units were PANs and the HFNs and TANs were much less frequent in our sample. On the other hand, neuron type distribution in the anesthetized cats differed from the distribution that we observed in the awake cats. Interestingly, not the PAN was the biggest group in this sample. It seems to be that we have lost the majority of these neurons in the anesthetized animals. The biggest population of the recorded CN neurons from anesthetized feline model were HFN, PANs were less represented in the anesthetized cats and even fewer neurons were found to be TANs.

The differences between the two feline models are also evident in case of LFP changes during visual stimulation (dynamic and static stimulation). The vast majority of the LFPs recorded in behaving cats showed significant activity changes at least in one visual condition and one frequency band, whereas in the anesthetized cats the visually evoked LFP changes were less frequent.

In the awake, behaving model the proportion of visually evoked LFP power changes were observed to be higher in all investigated frequency band except gamma (delta, theta, alpha, beta) compared to the results found in the anesthetized cats. However, the difference is much more obvious in case of static stimulation in the alpha and the beta frequency bands and concerning dynamic stimulation to the theta, the alpha and the beta bands.

These results indicate that halothane anesthesia has an obvious suppressive effect on the cat CN. Halothane anesthesia significantly decreased not just the background activity and visually-evoked responsiveness of NC neurons but significantly suppressed the visual-evoked LFP power alterations as well. The entire PAN population was lost in the anesthetized model. However, these differences are less obvious concerning the low frequency signals, LFPs.

Summary

Our results demonstrated that halothane anesthesia has an obvious suppressive effect on the feline CN. There was a significant difference not only between the single-cell neuronal activities but also between the visually evoked LFP changes recorded from the feline CN in an anesthetized and a behaving model. Halothane anesthesia decreased the number of visually responsive CN neurons and reduced strongly their background activity so much that. the analysis of the biggest neuronal population, the PANs (possibly corresponds to medium spiny neurons) was almost impossible, because of their extremely low activity. We demonstrated that there were less but still obvious differences between the visually evoked LFP changes in the CN of the two feline models. Anesthesia significantly suppressed the significant LFP power changes to applied visual stimulation. Based on the presented results we suggest the benefits of the awake animals in the electrophysiological investigation of the CN. The work with awake and behaving animals in visual electrophysiology seems to be much more difficult than the work with anesthetized animals, however, the results from the awake behaving animals are much more evident both in the single cell activities as well as in the visually-evoked LFPs.

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List of publications related to the subject of the thesis

I. Barkoczi B, Nagypal T, **Nyujtó D**, Katona X, Eordegh G, Bodosi B, Benedek G, Braunitzer G, Nagy A Background activity and visual responsiveness of caudate nucleus neurons in halothane anesthetized and in awake, behaving cats, Neuroscience 356 (2017) 182-192
IF: 3.382

II. **Nyujtó D**, Kiss A, Bodosi B, Eördögh G, Tót K, Kelemen A, Nagy A Visually evoked local field potential changes in the caudate nucleus are remarkably more frequent in awake, behaving cats than in anesthetized animals. Physiol Internat in Press (2023)
IF: 1.4

Other publications

I. Puszta A, Katona X, Bodosi B, Pertich Á, Nyujtó D, Braunitzer G, Nagy A Cortical Power-Density Changes of Different Frequency Bands in Visually Guided Associative Learning: A Human EEG-Study. Front Hum Neurosci. 12 (2018) 188
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II. Puszta A, Pertich Á, Katona X, Bodosi B, Nyujtó D, Giricz Z, Eördegh G, Nagy A Power-spectra and cross-frequency coupling changes in visual and Audio-visual acquired equivalence learning. Sci Rep 9 (2019) 9444
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III. Puszta A, Pertich Á, Giricz Z, Nyujtó D, Bodosi B, Eördegh G, Nagy A Predicting Stimulus Modality and Working Memory Load During Visual- and Audiovisual-Acquired Equivalence Learning. Front Hum Neurosci 8 (2020) 569142
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IV. Eördegh G, Pertich Á, Tárnok Z, Nagy P, Bodosi B, Giricz Z, Hegedűs O, Merkl D, Nyujtó D, Oláh S, Őze A, Vidomusz R, Nagy A Impairment of visually guided associative learning in children with Tourette syndrome. PLoS One 15 (2020) e0234724.
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