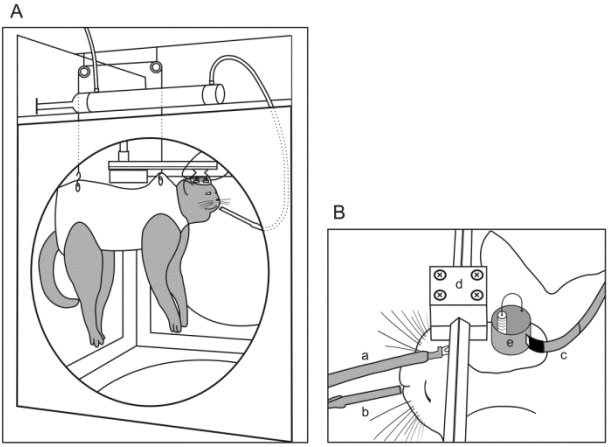
Visual response characteristics of neuronal clusters in the caudate nucleus of behaving cats

PhD thesis



Dr. Tamás Nagypál

Department of Physiology

Faculty of Medicine, University of Szeged

Szeged

2015

Introduction

The caudate nucleus (CN), the main input structure of the basal ganglia, is one of the subcortical brain structures, which is in the focus of neuroscience research. Electrophysiological researches, which were performed in the last 50 years, have confirmed the central role of the CN in regulation of sensorimotor and cognitive processes such as goal-directed action, memory functions, learning methods, sleep and emotion. It is also assumed that for the eliciting of the normal motor behavior the sensory information from the environment to the basal ganglia is essential. It is known that CN neurons are sensitive to various modalities of visual stimulation, i.e. static and dynamic visual components are also represented here. CN neurons were strongly sensitive to very low spatial and from intermediate to high temporal frequencies and possessed narrow temporal and spatial frequency tuning. Neurons with these spatio-temporal visual response properties have all the capacities to perceive optic flow and are good candidates for tasks involved in the perception of motion and probably in the perception of changes in the visual environment during self-motion. The questions raise whether the optic flow stimulus can directly activate the CN neurons and what kind of visual neuronal responses can be found in the CN of the feline brain.

Since the 1960s, striatal neurons were aimed to classify based on their firing patterns in two big groups: phasically active neurons (PANs) and tonically active neurons (TANs). In the middle 1990s, there were reliable reports on the third distinct subtypes of striatal neurons the high firing (HFN) GABAergic interneurons that could be further divided on the basis of their neurochemical properties in different subpopulations. Based on the results of the rodent and primate studies there is strong overlap between the anatomical and electrophysiological clustering of the CN neurons. PANs overlap strongly with the

medium spiny projection neurons, HFNs overlap with parvalbumin immunpositive GABAergic interneurons and the TANs seem to build the same cluster as the cholinergic interneurons. The questions rise whether there is the same electrophysiological clustering of the feline caudate body? Can we group the striatal neurons of the cat according their electrophysiological properties?

In the last few decades, behaving animal models have gradually gathered ground in neurophysiology, due to their advantages over anesthetized, paralyzed models. Behaving animal models are often used in primate visual experiments, but they have rarely been utilized in cats because of technical difficulties. In visual electrophysiology, the investigated structures often exhibit visuomotor activity (e.g. saccades). This makes a continuous monitoring of eye movements indispensable. In order to exclude the effects of eye movements the cats they have to maintain fixation during electrophysiological recordings and the eye position of the animal has to control continuously. In our laboratory we have been investigating for decades the sensory properties of the basal ganglia and the connected ascending tectofugal visual system. We have hitherto performed our experiments on anaesthetized and paralyzed cats. In order to exclude the effects of the anesthetics we have aimed to develop a feline model, which would be suitable for chronic visual and multisensory electrophysiological recordings in the awake, behaving eye-movement controlled cats.

Aims of the study

The aims of the thesis were to examine and evaluate the role of the CN of the awake, behaving cat in visual information processing. The primary goal of my thesis is to establish a new, behaving, head restrained and eye movements control

feline model for chronic visual experiments. The secondary goal is to categorize the neurons of the feline CN according their electrophysiological properties in different functional clusters. Furthermore, we aimed to analyze the responsiveness of the CN neurons in a visual fixation paradigm to static as well dynamic (optic flow) visual stimuli.

The specific aims were the following:

1. To establish a new, behaving, head restrained and eye movements control feline model for chronic visual experiments.
2. To classify the CN neurons according electrophysiological properties.
3. To describe the visual response characteristic of the CN neurons to static and dynamic visual stimuli.
4. To compare the response characteristics of neuronal functional groups of the CN.

Materials and methods

The animals were adapted to the laboratory environment and their temper was also observed. Once the cat got accustomed to the laboratory environment, it was carefully clothed into the canvas harness. This harness leaves the head, tail and legs free. Initially, the cat spent only a few minutes in the harness, which was extended to two hours. The next step in training, which is a novelty of our model, was the suspension of the animal in the experimental stand. The experimental stand is a cubical structure with each side open, in which the suspension harness is fastened at two points in by a rope pulley block. In the following step, the head of the suspended cat was fixed to the stereotaxic frame by the implanted steel headholder with two stainless steel bars. In our model, the cats have to tolerate a canvas bag around their body, a suspended position and

restrained head. The implanted reclosable plastic recording chamber and microdriver system allowed a stable recording background from the feline CN. In order to monitor the eye movements of the animals, a scleral search coil was implanted into the eye.

The animals had to learn the visual behavioral task. The initial part of behavioral training concentrates on fixation. If the cat holds fixation for a pre-set duration within the acceptance window, it receives food reward. During the fixation training, the fixation time was gradually increased from 100 ms to 1500 ms. The size of the initial fixation window was ±10 degrees for both cats. During the training period, it was reduced to ±2.5 degrees in ±2.5 degrees steps. This was the final size of the fixation window in the case of both cats. After the fixation training, either random dot pattern (static) or optic flow (dynamic) stimuli were applied, during the animal maintained fixation. The size of the dots was 0.1^{o} in diameter and their speed increased 0 to 7^{o}/sec toward the periphery. If the animal started the trial (put practically its eye within the fixation acceptance window and held there until 500 ms) visual stimulation was performed. Firstly random dot pattern (stationary visual stimulus) and then optic flow (dynamic visual stimulus) stimuli were applied.

Extracellular multielectrode recordings were performed with eight implanted parylene isolated platinum-iridium wire-electrodes (diameter: 25 μm) from the first cat and with eight implanted formvare insulated nickel-chrome wire-electrodes (diameter: 50 μm) from the CN of the second cat. In order to separate phasically and non-phasically active neurons of the CN the proportion of long interspike-intervals (ISI) to all ISIs was introduced (PropISI>2sec). Post-spike suppression was calculated, too. Based on these properties the striatal neurons were separated into three categories (PFNs, TFNs, and HFNs) based on $PropISI_{>2sec}$ and the length of the post-spike

suppression. For each unit two autocorrelograms (with ±100 msec and with ±1 sec windows), an ISI plot and average firing rates were calculated. PANs could be characterized by the occurrence of some long (> 2 sec) ISIs in addition to phasic activity with short ISIs, putative fast-firing neurons by high average firing rates and a lack of ISIs > 1 sec, and putative tonically-firing neurons by wide central valley in the autocorrelograms and spiking at 2-10 Hz.

Results

Developing a behaving, head restrained and eye movements control feline model

We have developed a new chronic feline model, which is suitable for electrophysiological recordings from visual brain structures. The most straightforward points of the model are the daily at least two hours recording time, the several years recording period from the same animal, the continuous eye control and the restrained head of the suspended animal during the experiments. These abilities make this model ideal for classic visual electrophysiological experiments. In order to exclude the effect of eye movements on the recordings of neuronal activities the head restrained, cats had to be able to maintain their fixation. The final result of the training is that the head restrained, suspended animals can fixate quite accurately (within a ±2.5 degree fixation acceptance window), even during dynamic visual stimulation. A continuous control of eye movements was an essential part of our experiments. By the eye-tracker method, it is possible to follow and exactly reconstruct (visualize) the eye movements of the animals. This also enabled us to detect failed fixation, in which cases the trial was aborted.

Classification of the CN neurons

In the applied behavioral visual fixation paradigm altogether 346 neurons were recorded from the dorsolateral part of the CN. Based on the above mentioned electrophysiological properties the recorded neurons from the feline CN were divided in three clusters: PANs (221 neurons), HFNs (88 neurons) and TANs (28 neurons).

PANs have peak autocorrelogram and ISI values were over 2 seconds, too. The $propISI_{>2sec}$ is usually higher than 0.5 and the spontaneous discharge rate is low in most cases under 3 spike/sec. The HFNs have blunt peak autocorrelograms, the $propISI_{>2sec}$ is lower than 0.5 and the spontaneous discharge rate is higher than 5 spike/sec. The big, deep gap in the autocorrelogram is typical for the TANs and the $propISI_{>2sec}$ is lower than 0.5. Spontaneous discharge rate of the tonically active neurons is between 2 and 12 spike/sec. Because of the very low spontaneous discharge rate (below 1 spike/second) we excluded 135 phasically active putative projection CN neuron from the analysis. Further nine CN neurons were excluded from the analysis, while we were unable to classify satisfactory them in each of the above mentioned three functional groups.

Visual responses of neuronal subtypes of the feline CN

The CN neurons showed mainly increased firing rate while in some cases decreased activity was also found during the visual behavioral paradigm. Significant changes in the activity of the CN neurons were recorded not only during stationary and dynamic visual stimulation. According to earlier results reward-related neuronal responses were also recorded shortly before and during the reward period after the correct completion of the task.

Response characteristics of the PANs

After the excluding of the neurons 86 PANs were analyzed during the fixation paradigm. The mean spontaneous discharge rate was 2.93 spike/sec (SD: ± 2.18 spike/sec). Overall, the visual responses of the PANs were moderate or weak. During stationary visual stimulation 50 neurons showed significant change in their activity. In 26 cases this meant a significant increase, and in 24 cases a significant decrease was seen. 29 neurons showed activity change during dynamic visual stimulation. During the 'center out' optic flow 17 of them and during 'center in' optic flow 16 of them showed increased activity. On the other hand 8 of the PANs showed decreased responses to optic flow. In the reward phase of the paradigm 27 neurons showed significantly increased activity and six of them posessed decreased discharge rate. The question raises whether this activity is clear reward-related and/or not related to the offset of the stimulus. In order to check this we have analyzed the aborted trials, too where the animal had broken the fixation. In this case the stimulus disappeared immediately and the animal got no reward. If we aligne the discharge of a neuron to the offset of the stimulus and if the response is reward-related no peak in the PSTH can be observed. If there is a peak in these histograms from the aborted trials after several 10 ms after the dissappearing of the stimuls the activity is related to the offset of the stimulus. Only one PAN was sensitive to offset of the stimulus.

Response characteristics of the HFNs

In the paradigm 88 HFNs were analyzed. The mean spontaneous discharge rate was 14.45 spike/sec (SD: ±6.81 spike/sec). During the stationary phase of the paradigm 18 neurons possessed significantly increased and 18 neurons showed significantly decreased activity. 37 high-firing CN

neurons responded to optic flow stimulus. During the ‘center out’ optic flow 16 neurons showed and during ‘center in’ 20 of them possessed increased activity. The visual responses of this group is much clearer and stronger than those of the PAN and TANs. During the reward phase of the paradigm 30 neurons increased their activity and none of the HFNs possessed significantly decreased activity. The analysis of the responses to the disappearing of the stimulus showed that 8 of the analyzed HFNs were active during the offset of the stimulus.

Response characteristics of the TANs

Beside the PANs and HFNs a low number of CN neurons (28) were classified in the TAN group. The mean spontaneous discharge rate was 5.24 spike/sec (SD: ± 2.37 spike/sec). During stationary visual stimultaion 11 neurons showed significantly increased and 2 decreased activity. Seven of them responded with increased discharges to optic flow stimulus. The visual responses of the TANs were moderate or weak. During the reward phase of the paradigm 6 neurons posessed increased activity. The offset of the stimulus modified the activity of one TAN.

Sensitivity to the direction of the optic flow

Altogether 74 (30 PANs, 36 HFNs and 8 TAN) of the 346 analyzed CN neurons showed significant activity change to optic flow stimuli. In order to check the direction sensitivity of the units, the responses to ‘center in’ and ‘center out’ optic flow of each optic flow sensitive CN neuron was compared. The majority of the CN units were unable to distinguish in their discharge rate between the ‘center in’ or ‘center out’ direction of the optic flow stimulus. However, twenty of them (27%) were sensitive to the direction of the optic flow stimulus nine of these units belong to the PANs and eight to the HFNs and three to the TANs. In the direction sensitive

group the neuronal responses to ‘center in’ optic flow were compared to the responses to ‘center out’ optic flow stimulus. 9 CN units showed significant stronger responses to ‘center out’ and eleven to ‘center in’ optic flow. In population level there was no significant difference between the proportion of direction preferences within PANs, HFNs and TANs (Chi-square test; $\chi^2(2)= 1.001$, df=2, level of significance: 0.05). Thus the PANs, the HFNs and the TANs of the CN could code the direction of the optic flow in the same manner.

Activity of neuron groups during different phases of the behavioral paradigm

We have analysed the neuronal responses to random dot pattern and ‘center in’ and ‘center out’ optic flow stimuli and compared the population activity of different CN groups using analysis of variance (ANOVA). The background activity of the HFNs were significantly higher ($p<0.001$) than that of the PANs and the TANs. To exclude the effect of the background activities we subtracted these from the whole activities and calculated the net firing rates. We have that applied the absolute values of the net discharge rates in the further analysis. The one way ANOVA analysis revealed that the net neuronal activities in all epochs of the applied behavioral paradigm i.e. stationary visual stimulation phase, dynamic visual stimulation phase, and reward phase were significantly different ($p<0.01$). The Tukey post hoc analysis revealed that the HFNs were most sensitive and possessed the strongest responses during the whole applied visual fixation paradigm.

Discussion

We have given the first detailed description about the activity changes/behaving of different CN neurons of the feline brain

in a visual fixation paradigm where static (random dot patterns) and optic flow dynamic stimuli were applied during continuous control of the eye-movements of the animal. To our knowledge this study is the first, which categorized the CN neurons of the feline brain according their electrophysiological properties in three big groups, PANs, TANs and HFNs and investigated the activity patterns of single-cells in each group to different kind of visual stimulation.

The first step of our work was to introduce a new feline model for chronic visual electrophysiology recordings. The long lasting behavioral training the eight implanted wire-electrodes and the implanted scleral search coil allowed us the recording of neuronal activities during visual stimulation from the CN of the feline brain.

Let's continue with one main novelty of the present study the electrophysiological categorization of neuron in the feline CN. We could divide on the basis of electrophysiological properties (background activity, shape of the autocorrelograms and prop$ISI_{>2s}$) of the CN neurons of the feline brain in three big groups. Similarly to earlier findings in rodents and primates PANs, TANs and HFNs were found in the feline CN, too. Earlier studies suggested the strong overlap between the three biggest anatomical (medium spiny, cholinergic and parvalbumin immunpositive GABAergic interneurons) and electrophysiological (PAN, TAN, HFN) groups of the CN neurons. PAN neurons overlap strongly with the medium spiny projection neurons, HFNs overlap with parvalbumin immunpositive GABAergic interneuronal cluster and the TFNs seem to build the same cluster as the cholinergic interneurons.

The other main novelty of the experiments presented here is the description of visual responsiveness of CN neuron of the feline brain in a visual fixation paradigm where the effect of static as well as dynamic visual information was investigated. The applied dynamic stimulus was quite new in the relation

with the CN while hitherto optic flow stimuli were not applied to check the striate neurons. The CN neurons were strongly sensitive to very low spatial and from intermediate to very high temporal frequencies and possessed narrow temporal and spatial frequency tuning. Neurons with these spatio-temporal visual response profiles could be good candidates to perceive optic flow. In the present study we checked this hypothesis and gave the first direct evidence on the processing of optic flow in the feline CN.

After the categorization of the CN units based on their electrophysiological properties we could investigate the visual response characteristic of each neuronal groups of the CN. Our results demonstrated that both the static and dynamic components of the visual information are represented in the CN. The majority of the CN neurons in each of the three groups (PAN, TAN, HFN) responded with increased discharge rates during the paradigm, while some units showed decreased discharge rate during different epochs of the paradigm. The PANs and TANs were more sensitive to static than dynamic visual stimulation thus the majority of them which possessed visual activity responded to random dot patterns and not to the optic flow. On the other hand the sensitivity of the HFNs was same to static and dynamic visual stimulus i.e the same amount of these neurons responded to random dot patterns and to optic flow stimuli. The stimulus offset modulated the activity of a significant population of HFNs but the disappearing of the stimulus could not influence the activity of the PANs and TANs of the CN. These response characteristics suggest that the PANs and TANs are primary sensitive to static continuous, unchanged visual stimuli. The response characteristics of the HFNs are different because beside the sensitivity of much units to static stimuli the majority of these neurons were responsive to the changes of the visual environment, i.e. to movements and to offset of the stimulus. Furthermore the net responses of the HFNs were significantly

higher than those of the PANs and TANs during each epoch of the whole paradigm. These suggest that HFNs are the most sensitive units in the CN to visual stimuli.

We have also investigated whether the direction of the optic flow (center in or center out) can modify the activity of the CN neurons. The majority of the CN units in each group were unable to distinguish in discharge rate between the 'center in' or 'center out' direction of the optic flow stimulus. On the other hand twenty CN neurons (nine PANs, eight HFNs and three TANs) were sensitive to the direction of the optic flow stimulus. In this group about half of the selective neurons (11 neurons) responded stronger to center-in stimulus while the second half of them (9 neurons) responded stronger to the center out optic flow. These results of the direction sensitive CN units support the earlier hypothesis that the CN neurons are good candidates for tasks involved in the perception of motion and probably in the perception of changes in the visual environment during self-motion.

In summary, we have demonstrated the application of our head restrained eye-movement controlled feline model in visual electrophysiological experiments where visual responsiveness of CN neurons were analyzed in detailed. We have demonstrated that different CN neuronal groups (PAN, TAN, HFN) are differently sensitive to static and dynamic visual stimulation. PAN and TAN neurons are primary sensitive to static stimuli while HFNs are primary sensitive to changes in visual environment of the animal. Furthermore we have given the first evidence on optic flow processing in the feline CN and suggested the role of the CN in motion detection.

Acknowledgements

I respectfully thank to Dr. Attila Nagy who was my mentor and supervisor during my PhD work. Special thanks to Professor Dr. György Benedek, for his helpful and instructive guidance. I express my gratitude to Professor Dr. Gábor Jancsó for allowing me to participate in the Neuroscience PhD Program. My special thanks go to Dr. Péter Gombkötő and Dr. Antal Berényi for their help in laboratory work and in data analysis and their friendship. I am also grateful to Dr. Gábor Braunitzer for his critical review of the drafts and the final version of this thesis. I would like to acknowledge the help of Dr. Alice Rokszin, Györgyi Utassy, Robert Averkin, Tamás Puskás, Anett Nagy, Dr. Mihály Vöröslakos, Dr. Attila Őze, Dr. Balázs Barkóczi and Dr. Balázs Bodosi.

I express my most sincere gratitude to Gabriella Dósai for her valuable laboratory assistance. Many thanks go to Péter Liszli for his expert help in solving hardware and software problems. I would like to express my thanks to all of my colleagues and friends in the Department of Physiology for their support and kindness. It has been nice to work with them in this department.

My deepest thanks go to my parents for their continuous love and help in my life.

I would like to express my extra gratitude to the Talentum Foundation of the Gedeon Richter Plc. for their financial support.

List of publications

I. Nagypál T, Gombkötő P, Utassy G, Averkin RG, Benedek G, Nagy A (2014) A new, behaving, head restrained, eye movement-controlled feline model for chronic visual electrophysiological recordings. J Neurosci Methods 221:1-7.
IF: 2.025

II. Nagypál T, Gombkötő P, Barkóczi B, Benedek G, Nagy A (2015) Activity of Caudate Nucleus Neurons in a Visual Fixation Paradigm in Behaving Cats. PLoS ONE 10(11): e0142526.
IF: 3.234

III. Gombkötő P, Berényi A, Nagypál T, Benedek G, Braunitzer G, Nagy A (2013) Co-oscillation and synchronization between the posterior thalamus and the caudate nucleus during visual stimulation. Neuroscience 242:21-27.
IF: 3.357