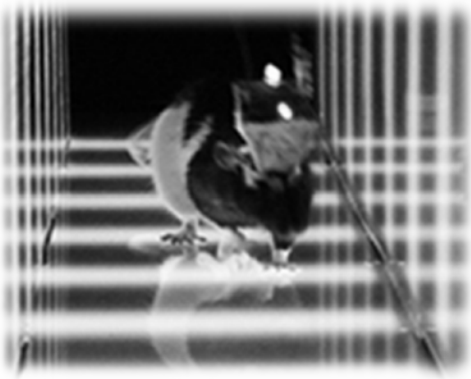
Investigation of visual motion sensitivity in the striatum of freely moving rats

Summary of the doctoral thesis



Anett Nagy

Department of Physiology
Faculty of Medicine, University of Szeged

Szeged

2020

Introduction

To accommodate the ever-changing demands of survival and to maintain homeostasis, nervous systems must effectively utilize sensory information when choosing the appropriate motor actions from the available repertoire. The striatum (also called as caudoputamen, CPu) may play an important role in this sensory-motor translation, as it integrates massively converging inputs from virtually all cortical areas and many subcortical structures, and serves multiple aspects of cognition such as decision-making, reward prediction and action planning. The CPu processes information in a very distinct way from the cortex. The internal circuitry of the CPu is shaped almost exclusively by GABAergic inhibitory neurons and a few cholinergic neuromodulator cells, almost all receiving cortical inputs.

Ninety-five percent of these GABAergic cells form the more or less anatomically homogenous group of medium spiny neurons (MSNs) and are traditionally identified as phasically-firing neurons (PFNs) in extracellular electrophysiological recordings. Cholinergic interneurons make up only 0.5-1% of the striatal neurons, but act as massive integrators; while they receive extensive glutamatergic excitation, they are also modulated by serotonergic, noradrenergic and dopaminergic inputs. As they maintain a relatively stable firing rate, traditionally, they are classified in the electrophysiological recordings as tonically firing neurons (TFNs). The remaining five percent of aspiny GABAergic cells were recently described as a mixture of at least eight morphologically, neurochemically and electro-physiologically different classes of cells. Determining the exact type

of these neurons based purely on extracellularly recorded electrophysiological features of their spike trains is almost impossible. They are grouped together as fast-firing interneurons (FFNs), based on the properties of the parvalbumin (PV) expressing fast-spiking interneurons, which class dominates this group. The electrically coupled network of these FSIs act as filters, because they transduce information only if they receive multiple synchronous inputs and can precisely control spike timing in MSNs via $GABA_A$ signaling at perisomatic synapses.

Although the principles of local connectivity of the striatal neuronal classes are preserved throughout the whole striatum, detailed functional studies revealed that still there is a well-expressed functional segregation within the CPu. While the ventral part is mainly associated with reward and motivation related processes (e.g., reinforcement learning), the dorsal striatum is involved in executive functions based on stimulus-response learning. The cooperation of the mediodorsal CPu and the hippocampus promotes action selection based on learned cognitive-spatial information, especially in cases where competitive cues are present. The CPu likely operates on a complex, preprocessed representation of a multisensory environment.

Detecting moving objects in a calm environment when the sensory representation of the latter is not steady either has high importance; it can guide movements when reaching for prey or trying to avoid a predator. The striatum is a candidate to support such decisions. Motion or salient elements and stationary features of a visual scene are dissociated at very early stages of the visual system;

however, both representations are simultaneously present in the CPu. Distinguishing whether the visual dislocation of a perceived image happens due to the observer's self-motion or due to a moving object indeed, is crucial to guide the behavior properly. Evolution offers a well-conserved solution; monitoring a corollary discharge - an internal copy - of the motor commands helps to filter out the sensory consequences of a self-action. It is unknown yet if a similar mechanism is in place for the recognition of moving objects and related action selection.

Network-level investigation of these functions requires large number of neurons to be simultaneously observed. High-density, microelectrodes ('silicon probes') offer the possibility to perform large-scale recordings at high temporal and high spatial resolutions. While nanomachining offers ever-larger recording site numbers, its use in freely moving animal experiments is constrained by the burdens of the recording electronics. In contrast, experiments on restrained animals are not suitably designed to investigate the self-motion induced components of the visual motion perception, since sensory feedbacks of the self-motion alter the activity of these networks.

As part of my doctoral work, we set out to overcome this obstacle by developing a high throughput integrated microelectronic system, including high-density, multiple-shank recordings of unit activity and local-field potentials (LFP) from multiple brain regions in freely moving rodents. I utilized this technology to investigate the integrative capacities of the striatal neuronal networks, with special interest on self-induced and self-independent motion processing.

Aims of the study

My doctoral work focused on examining the response characteristics of the dorsal CPu neurons to various visual experiences modulated by self-induced and self-independent motion percepts. Here, I summarize my experimental work to decipher 1) which aspects of visual motion information are reaching the caudate nucleus, 2) what is the origin of these inputs, and 3) how these inputs shape the activity of striatal neurons. My specific aims were the followings:

- To identify the anatomical localization of visual motion-sensitive neurons in the CPu and to validate our novel high-density recording technique during visual stimulation and free behavior.
- To develop experimental approaches to control visual motion percepts in unrestrained animals.
- To quantify the sensitivity of the dorsal CPu neurons to self-independent and self-induced visual motion in freely moving rats.
- To identify the possible sources of the striatal visual information by tract-tracing, immunohistochemical methods, and electrical stimulation, and to describe the role of this pathway in shaping the visual-motion related visual responses

Materials and Methods

32 rats (Long-Evans, 3-12 months old) were used in this study. Initially, anterograde and retrograde tract-tracing of corticostriatal neurons were performed by injecting 10% biotinylated dextran amine (BDA) into the secondary visual cortex (V2) and 4% Fluoro-Gold into the dorsomedial striatum, respectively, in altogether 13 anesthetized

rats. After one-week survival, the rats were transcardially perfused, coronal brain sections were prepared, and processed for immunohistochemistry with primary and secondary antibodies. Nine rats underwent an experiment to induce striatal cFos, an immediate early-gene expression by visual stimulation along with the anterograde tracing. One-week after BDA injection, rats were reared in complete darkness overnight, and then five of them were exposed to various moving visual stimuli displayed on the walls of their home-cage. After that, their brains were processed for cFos, BDA and PV immunohistochemistry. Sections were then mounted, counterstained, coverslipped and investigated using confocal microscopy. Evaluation of tile-scans were performed using semiautomated quantification.

Awake electrophysiological experiments were carried out using movable, implanted 32 and 64 channel silicon electrodes. A copper mesh was attached to the skull and connected to the ground to act as a Faraday cage. Probes were moved gradually in 150 µm steps per day until the desired recording positions were reached. Neuronal recordings were performed daily, with concurrent recording of the animals' position and behavior using a synchronized camera system.

To reduce the volume and weight of the instrumentation between the electrodes and the recording equipment, we used 32-channel analog signal multiplexers explicitly developed for brain recording applications with custom made processing electronics and an ultra-flexible polyimide-based interconnect cable. Each of the electrical signals acquired by the silicon probe were amplified and band-pass filtered. The broadly tuned cutoff frequencies allowed the

recording of broad-band signals (LFP and unit activity simultaneously) from all recording sites.

To expose the animals to self-motion incongruent visual motion percepts they were placed in a home cage-like visual stimulating environment with walls projecting various visual stimuli, while the animals were freely observing their environment. Each stimulus trial consisted of an initial one-second isoluminant gray screen, one second of full-screen stationary grating with sinusoidally modulated luminance in a given orientation (eight orientations), and with a given spatial density (170, 85, 42.5 mm/cycle), and finally one second of moving grating, where the grating was sliding orthogonal to its orientation with a given temporal velocity (1200, 600 and 300 mm/s). A pseudorandom sequence of visual stimulus trials was generated by random combinations of orientation and spatial/temporal properties. The activity of CPu and visual cortical neurons were recorded during the visual stimulation. In animals with stimulating electrodes in the visual cortex, we also tested single unit and evoked potential responses in the CPu for cortical electrical stimulation.

To investigate the responses of CPu neurons to visual motion induced by the self-motion of the animals, they were running for water rewards in the linear maze made of translucent walls and floor. Using a dual mirror system, stationary striped patterns were projected on the walls of the maze to let the rats' motion induce visual motion percept. As control, walls were set to uniform grey. Neuronal spikes were detected (Spikedetekt2), automatically sorted (KlustaKwik2) and their clusters were manually adjusted (KlustaViewa). Striatal neurons were

classified as reported by Schmitzer-Torbert and Redish in 2008. Cross-correlation analysis has been applied to detect putative monosynaptic connections. Peristimulus time histograms and rate maps were constructed from the action potential time-series, triggered by the onsets of visual stimulation sequences or the spatial positions. Firing rates and modulation indices were calculated for the periods during uniformly grey, stationary grating and moving grating stimulation, and their combinations, respectively.

Power spectrum and time-resolved spectral analysis were performed on the low-pass filtered LFP signals. Spectra were whitened. LFP-phase histograms were constructed by taking the instantaneous LFP phase values corresponding to the spikes. Granger causality and time and frequency-resolved cross-correlation were estimated for each V2 and CPu signal pairs. For statistical testing, Student's t-test, Wilcoxon rank-sum test, or Kolmogorov-Smirnov tests were used, with Bonferroni correction for multiple comparisons.

Results

Dorsomedial striatum receives innervation from the visual cortex

By retrograde tracing with Fluoro-Gold, we found that the neurons of the most rostral parts of the primary and secondary visual cortices (V1 and V2) sent strong projections to the dorsomedial CPu. Based on their morphology, the labeled neurons are putative pyramidal cells. We did not find labeled neurons at the caudal parts of the V1 nor at the lateral geniculate nucleus, however, the lateral-posteromedial and posterior nuclei of the thalamus were densely

labeled. Confirmatory anterograde BDA tracing and visual stimulation confirmed that axons originating from V2 neurons colocalized with cFos+ striatal neurons and were restricted to the mediodorsal part of CPu. Control experiments confirmed that the cFos expression was a result of the visual stimulation.

Large-scale extracellular recordings by multiplexed acquisition

To analyze the intrastriatal connectivity and the information transfer from the cortex to the striatum, we decided to perform large-scale extracellular recordings using multishank silicon electrodes. To validate the newly developed recording system, we compared the signal quality to a commercially available recording system, serving as industrial standard. The input referred noise of the system was comparable to the reference system's noise level. The common noise component of the signal was significantly lower, the full range amplitude was approximately 20% smaller and the single unit yield was more than triple than that of the control system. Using this implementation, we could successfully demonstrate the feasibility of long-term 256 and 512 channel recordings in freely moving rats.

Identity and connectivity of the recorded striatal neurons

We recorded the activity of altogether 734 neurons from unrestrained rats performing various behavioral and visual tasks. 64 %, 22 % and 8 % of all recorded neurons were classified as PFNs, FFNs and TFNs, respectively. In six percent of the recorded neurons, the classification was ambiguous and were discarded. The overrepresentation of FFNs compared to the anatomical proportions

was attributed to the less reliable classification of the more meagerly firing PFNs in short sessions. The recorded neurons were sparsely connected. Our cross-correlogram-based analysis revealed a relatively large number of electrical synapses between fast-firing neurons (6.77 % of all theoretically possible pairs), and a monosynaptic excitation exerted by the TFNs on FFNs (4.84 %), but not on PFNs. The very few detected inhibitory interactions were caused by FFNs.

Striatal responses to self-independent visual stimulation

Twenty-five of 289 neurons were considered responsive to passive, self-motion independent visual stimulation (pVis+ neurons). They were more sensitive to broader stripes sliding with high velocity but showed no systematic direction preference. The pVis+ neurons either increased their firing rate in the presence of the stationary grating, became inhibited by the moving gratings or the combination of these two. Most of the pVis+ and almost none of the pVis- neurons could be entrained by electric stimulation of the visual cortex (El+ neurons). Entrainment involved both monosynaptic and polysynaptic responses. The majority of both pVis+ and El+ neurons were FFNs.

Striatal responses to self-congruent visual stimulation

325 of 685 CPu neurons displayed a significant change in their firing rates when running in the striped environment. The distribution of these aVis+ cell types resembled the anatomical proportions. Importantly, all pVis+ neurons were also responsive in this task; thus, the pVis+ cells can be considered as a subgroup of the aVis+ neurons. Only 23 of the aVis+ neurons modulated their firing rates as a function

of the instantaneous running speed. In agreement with their behavior during the passive visual stimulation experiment, pVis+ neurons decreased their firing rate during running in the striped linear maze. The visual modulation of the aVis+ neurons was similar to the response profile of the pVis+ neurons, however, their presence was not restricted to the mediodorsal segment of the CPu.

LFP phase preference of the recorded striatal neurons

Neurons of the CPU were generally phase-locked to the characteristic oscillations of the surrounding local field potentials. We found that theta and beta powers were significantly weaker at recording locations where pVis+ neurons were present, while delta and gamma band powers generated by local networks were similar at all locations. In general, the pVis+ and aVis+ neurons were more weakly coupled to oscillations than the pVis-/aVis- ones, and consistently with the weaker LFP power, this difference was more prominent in the theta band. Phase preferences of pVis-/aVis-, aVis+ and pVis+ neurons were similar at all five frequency bands.

Phase, space and reward coding of the recorded neurons

A significant number of neurons showed spatial location selective activity with one single place field, similarly to the hippocampal place cells at CA1 region. The presence of the striped pattern altered the firing rates but did not influence place coding. The strength of the modulatory effect of stripedness on neurons was similar in both running direction. Similar to hippocampal place cells, a subset of the recorded neurons also possessed theta phase

precession. More than half of the recorded neurons expressed some reward-related change in their activity. These findings are similar to the properties of ventral striatal neurons. Overall, the pVis+ neurons were more likely to express place-field-like activity, phase precessing, or reward-related activity than their pVis- peers.

Information transfer between the visual cortex and striatum

As it is almost impossible to reveal long-range monosynaptic connections in intact animals using electrophysiology, we had to restrict our analysis regarding the functional corticostriatal cooperation to local field potential investigations. The frequency spectra of the LFPs recorded at the CPu and at V2, and their coherence were identical in striped and blank trials, suggesting that the visual environment does not substantially alter information transfer between these structures. We employed a novel time- and frequency-resolved cross-coherence estimation, which revealed that V2 drives CPu with transient, mid-gamma band oscillations that become significantly weaker in the striped maze. This suggests the presence of a population level communication 'channel' influenced by the visual environment.

Discussion

The main conclusion of my work is that in addition to the small distinct group of striatal neurons that are responsive to both self-motion independent (passive) and self-motion induced (active) visual stimuli, there is a larger subset of striatal cells that only sense self-motion induced visual changes. Consequently, it is essential to investigate visual motion-related sensory processing in freely moving

animals, instead of in anesthetized or head-fixed preparations. The perceptual capacity represented by these two cell groups may be important for survival, as they allow the recognition of incongruently moving objects (such as prey) in a visual environment, which is continuously changing by itself due to the self-motion of the observer.

Our results suggest that the striatum receives a preprocessed 'global percept' of the visual environment, instead of partial pixelated representations. We found that a possible source of this information may be the visual cortex, which is supported by earlier reports on a direct connection between the visual cortex and the dorsocaudal striatum in rats. This glutamatergic pathway was found to selectively innervate the matrix but not the striosomes, and to terminate mainly on MSNs. This cannot explain though why most of the pVis+ neurons were FFNs. FFNs also receive direct cortical inputs, but due to their high spontaneous firing rates, it is harder to detect an induced excitation. In addition, the observed inhibition of the FFNs exerted by visual stimuli suggests either an indirect cortical effect through other striatal interneurons (signal inversion) or an already negative coding scheme of the cortico-striatal afferents.

The anterolateral part of area 18a (lateral extrastriate cortex) of the mouse, which is the homolog of V2L and V2LM in the rat, is linked to the processing of self-motion cues, and expresses similar spatiotemporal sensitivity. The aVis+ or pVis+ cells did not respond to blinking light stimulation at all, which also suggests that the visual information may originate from a higher-order visual structure that is not sensitive to primitive on-off luminance transitions.

Our finding that almost all pVis+ neurons were excitable by electrical stimulation of the V2, strengthened by the gamma-band coherence of these structures that is modulated by the visual percept, suggests that a major source of the striatal visual information is rather cortical, but the lack of electrical excitability of aVis+ neurons also predicts a complementary subcortical input. A candidate subcortical pathway originates in the lateral-posteromedial nucleus of the thalamus, which projects to the same subregions of both in the CPu and the V2 that we investigated.

Interactions between multiple circuits of the brain are orchestrated by coherent LFP oscillations serving as ‘semaphores’, which are efficient filters for information transfer. We found that the neurons of V2 express transient oscillations in the mid-gamma band. Such patterns also appear in the CPu with approx. 20-30 ms delay, which is in good agreement with the latency between the striatal post-synaptic potentials and V1 evoked potentials after visual stimulation and matches the delay of the corticostriatal monosynaptic responses. It is possible that its purpose is to offer highly excitable time-slots when the CPu neurons become more sensitive to cortical information; however, it is doubtful whether these gamma patterns are related only to the visual cortical interactions, or multiple inputs are converging to the CPu during these short temporal windows.

The reward and space-related activity of the CPu is an integral part of the hippocampal, anteromedial prefrontal cortex, and the dopaminergic subcortical reward systems. The interaction of these inputs with the sensorimotor components provides a plethora of

information to associate places with reward and learn optimal survival strategies. Altogether the CPu should be considered as an integrator that has the chance to adjust procedural motor functions based on the internal representations of 'past experiences' (i.e., through hippocampal episodic and spatial memory) and the 'present circumstances' (i.e., through the contribution of sensory systems). Our findings regarding the simultaneous, conjunctive expression of sensory-related and contextual/spatial (e.g., hippocampal) features in the CPu may support this concept.

Conclusively, differential innervation distinguishes pVis+ cells from the larger pool of self-motion induced visual perceptors (i.e., aVis+ neurons) and establishes them as a separate functional group. I propose that the aVis+ neurons may provide robust feedback on the dislocation of the gait caused by the movements of the animal, while the successive comparison of the activity of aVis+ and pVis+ neurons can reveal the presence of independently moving objects in a non-stationary but neutral visual environment. This modality may be fundamental in selecting the appropriate action to challenge them.

Acknowledgement

I wish to express my sincere gratitude to Professors György Benedek, Gyula Sáry, Gábor Jancsó and Dr. Attila Nagy for providing me the opportunity to work in the Department of Physiology and the doctoral program. I acknowledge with thanks the kind of patronage, loving inspiration and timely guidance, which I have received from my supervisor Dr. Antal Berényi. I would also like to thank some of

the people who gave a hand in the creation of this work: Dr. Yuichi Takeuchi and Mari Takeuchi, their help in histology was indispensable while I was expecting my first daughter. This is the kind of help what one never forgets. I also wish to express my gratitude to Anikó Jász and Tamás Gyurkovics and other members of the Department of Physiology who rendered their help during the period of my project work. I am particularly grateful to Gabriella Dósai, whose assistance was essential during my early lab work.

Publications related to the subject of my thesis

I. **Nagy A.J.**, Takeuchi Y. and Berényi A. (2018) Coding of self-motion-induced and self-independent visual motion in the rat dorsomedial striatum.
PLOS Biology 16 (6), e2004712.

II. Berényi A., Somogyvári Z., **Nagy A.J.**, Roux L., Long J.D., Fujisawa S., Stark E., Leonardo A., Harris T.D., and Buzsáki G. (2014) Large-scale, high-density (up to 512 channels) recording of local circuits in behaving animals.
J. Neurophysiol. 111, 1132–1149.

Other publications

III. **Nagy A.J.,** Berényi A., Gulya K., Norita M., Benedek G., Nagy A. (2011) Direct projection from the visual associative cortex to the caudate nucleus in the feline brain.
Neurosci. Lett. 503, 52–57.

Cumulative impact factor and independent citations of the publications related to my thesis

IF = 11.000 (ISI); Independent citations = 128 (Scopus)

Cumulative impact factor of all publications

IF = 13.173 (ISI); Independent citations = 129 (Scopus)