

**The role of the medial prefrontal cortex
in learning and reward**

Ph.D. thesis

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Pécs, 2016

List of abbreviations

Acb: nucleus accumbens
AcbC: nucleus accumbens core
AcbSh: nucleus accumbens shell
ACe: the central amygdaloid nucleus
aCg: anterior cingular cortex
A-O: action-outcome
BSS: behavioral satiety sequence
BL: "before licking cluster"
BLA: basolateral amygdaloid nucleus
CMS: central motive state
CR: conditioned response
CRf: conditioned reinforcement
CTA: conditioned taste aversion
CS: conditioned stimulus
CS+: reinforced CS
CS-: not reinforced CS
DA: dopamine
ERN: error related negativity
LC: licking cluster
MD: mediodorsal thalamic nucleus
mPFC: medial prefrontal cortex
OFC: orbitofrontal cortex
PETH: perievent time histogram
PFC: prefrontal cortex
PIT: pavlovian-instrumental transfer
PL: prelimbic cortex
PSTH: peristimulus time histogram
s.e.: standard error of means
S-R: stimulus-response
UR: unconditioned response
US: unconditioned stimulus
VTA: ventral tegmental area

Introduction

One of the most important and interesting questions about brain function is learning, more precisely, what is learned in a certain situation, which brain regions are involved in a certain aspect of learning and how neuronal networks function during learning. The dissertation is about motivated, food (sweet tasting sugar solution) rewarded behavior. Motivation plays a central role in learning, in not learned and already learned (conditioned) behavior.

Endre Grastyán divided the history of motivation for three periods: the psychological, the neuropsychological and the incentive period (Grastyán, 1976). In the psychological period, which lasted from the 1910s until the early 1950s, motivation was considered an intermediate variable, which can be manipulated objectively by the experimenter (food deprivation in case of hunger). The first systematic theories of motivation and reinforcement appeared during this period: the drive reduction theory (Hull, 1943) and the law of effect (Thorndike, 1911). From the wording of the law of effect the response-reinforcing principle gradually became the main accepted basis for explaining behavioral modifications as a consequence of learning (Skinner, 1938; Hull, 1943; Skinner, 1953).

The second period, the neuropsychological one, began with the publication of the diffuse activating principle (Moruzzi and Magoun, 1949). According to Hebb's opinion "motivation is an engine, not a steering wheel" (Hebb, 1955). In this case motivation alone would be an aimless driving force, its direction would be determined by other mechanisms. Since the 1950s the incentive-motivational principle gradually became more important (Bolles, 1967; Bindra, 1968). Bindra invented the concept of the central motive state (CMS) and rejected the theory, that reinforcement is necessary for learning. The CMS is the result of a joint effect of organismic and environmental stimuli. According to Bindra, CSs gain incentive value triggered by incentive stimuli (US) and can influence the CMS like the US.

In the 1950s, experimental data obtained by using electrical stimulation of the hypothalamus led to the conclusion, that motivation is not only a hypothetical process, it has a neuronal substrate. One of the key discovery was the self-stimulation phenomenon by Olds and Milner, who investigated its effect on motivation in the rat hypothalamus (Olds and Milner, 1954).

In several experiments Grastyán studied the activation of the conditioned reflex (Grastyán és mts., 1956; Grastyán és mts., 1965a). Electrical stimulation of the hypothalamus elicited or inhibited CRs without the presence of CSs. By means of recordings of slow waves he demonstrated, that contra- or ipsiversive direct and rebound effects correlated with hippocampal theta activity (approach) or desynchronization (avoidance) respectively (Grastyán et al., 1965).

In one of the experiments described in this dissertation Pavlovian conditioning was used. During such a procedure the experimenter arranges contingencies between stimuli of the environment independently from the animals' behavior. A neutral environmental stimulus is paired with a biologically relevant unconditioned stimulus (US). The originally neutral stimulus becomes to be a conditioned stimulus (CS) and can elicit the conditioned response (CR, e.g. salivation) without the presence of the US (e.g. food). According to Pavlov's explanation an association is formed between the central representations of the CS and the US.

Beyond that, Pavlovian conditioning may create multiple associations. According to modern theories these associations are not the consequence of a unitary process (Wagner and Brandon, 1989). In the most simple case direct association is created between a CS and UR (S-R). S-R learning often occurs during overtraining in instrumental conditioning (Dickinson,

1994). A CS may activate a representation of the affect (fear or reward expectancy). Beyond that an association may be created between a CS and specific sensory properties of the US. Pavlovian conditioning may also create associations with emotions (e.g. fear) (LeDoux, 2000).

During instrumental conditioning the experimenter arranges contingencies between behavior of the animal and a reinforcing outcome (independently of behavior). Several psychological processes contribute to learning and instrumental performance (Dickinson, 1994). Behavior of humans and animals is often goal directed and can be flexibly modulated by motivation. Flexible representations are created in experimental animals during instrumental conditioning. Pavlovian CSs may modulate instrumental performance, this is the Pavlovian-instrumental transfer (PIT). In relation to Pavlovian conditioning another important phenomenon is the conditioned reinforcement (CRf). If a neutral stimulus is paired with a primary reinforcer, the stimulus gains affective or motivational value, therefore the animal works for the stimulus itself (Mackintosh, 1974).

The primary target of the dissertation was to unravel learning related mechanisms of the prefrontal cortex (PFC), more precisely the prelimbic part (PL) of the medial PFC (mPFC). The PFC, the amygdala and nucleus accumbens are parts of a neuronal network, which play central role in motivated behavior and learning.

The amygdala consists of several nuclei. In relation to emotional regulation the central nucleus (ACe) and the basolateral nucleus (BLA) are especially important. The amygdala has a central role in emotions (Klüver and Bucy, 1939). Lesions of the BLA or ACe cause impairments of the fear conditioning (LeDoux, 2000). The amygdala plays a role in the regulation of feeding (Lenard and Hahn, 1982; Lenard et al., 1982) and food rewarded conditioning (Hatfield et al., 1996). Its role in instrumental conditioning is less understood. Lesion of the BLA decreases the instrumental performance for conditioned reinforcers (Pavlovian CSs) (Cador et al., 1989).

Motivational effects of stimuli are partly organized by the nucleus accumbens (Acb). Different experiments have been performed about the DA innervation of the Acb, which has a prominent role in motivational effects of natural reinforcers and in drug abuse (Hoebel et al., 1983). The AcbC (core) is important for the locomotor approach response (autoshaping) (Parkinson et al., 2000) and PIT (Hall et al., 2001). The AcbSh (shell) mediates the motivational impact of unconditioned stimuli. Primary reinforcers like food or cocaine cause increased extracellular DA levels in the AcbSh (Bassareo and Di Chiara, 1999). In contrast CSs cause increased DA levels in the AcbC. The Acb has direct and indirect connections (via the amygdala and VTA) with the mPFC and can affect its function.

In rats the prelimbic cortex (PL), the anterior cingulate cortex (aCg) and the orbitofrontal cortex (OFC) are subregions of the PFC (Zilles and Wree, 1995). The first two regions are also parts of the medial prefrontal cortex mPFC. Each subregion of the PFC contributes to the regulation of motivated behavior.

A function of the PL is the detection of instrumental contingency (Balleine and Dickinson, 1998). The BLA may play a role in the central representation of incentive value. It was shown, that lesion of the connection between BLA and mPFC modulates instrumental choice (Coutureau et al., 2000). In rabbits (which have a PL similar to rats), in electrophysiological experiments, it was shown, that the PL contributes to Pavlovian conditioning (McLaughlin et al., 2002). A more recent paper demonstrated the role of the mPFC in Pavlovian trace conditioning (Pezze et al., 2015).

The aCg in primates and rodents has similar functions. Both respond to affective stimuli and control the function of different motor and autonomic systems. The rat aCg is important for guiding behavior if similar CSs predict different reinforcers. Neurons predicting unsigned

prediction error were detected also in the rat aCg in electrophysiological experiments (Totah et al., 2009).

Objectives

One of the most effective methods for study information processing in the brain is the single-unit recording in awake experimental animals. Numerous such experiments were performed during Pavlovian or instrumental conditioning. Despite of several data showing, that the mPFC (PL and IL) also plays a role in Pavlovian conditioning, its role in appetitive Pavlovian conditioning was investigated only in a few electrophysiological experiments.

Questions:

1. The PL plays a role in motivated behavior and food rewarded Pavlovian conditioning. However the electrophysiological correlates of these functions are not well understood. So in the first experiment we investigated the function of PL neurons on freely moving rats with implanted tetrodes. Rats were allowed to drink freely sugar solution (after drinking of water). Our question was how neuronal activity changes during free drinking of liquid food reward and whether responses are related to reward expectancy or consummation.
2. In our first experiment we found neurons which responded before drinking of sugar solution. We proposed, that these responses are related to reward expectancy. In order to investigate this assumption in more detail we applied in our second experiment the Pavlovian trace-conditioning in electrophysiological experiments in freely moving rats. In order to find out whether responses depend on movements toward the drinking tube or on reward expectancy, we measured head movements with an accelerometer mounted on the head of the rats. Our question in the second experiment was, how neurons in the rat mPFC respond to different phases of the Pavlovian trace-conditioning, and whether these responses code reward expectancy.
3. The mPFC (aCg and PL) receives motivation related inputs from the BLA and VTA. So, we investigated mPFC neurons in acute juxtacellular electrophysiological experiments, during electrical stimulation of the BLA and/or VTA. Neurons were labeled with Neurobiotin which was visualized by histochemical methods (ABC-peroxidase, Ni-DAB, Ag intensification). We examined the relation between morphological and electrophysiological characteristics of different types of neurons. We also investigated whether neurons receive convergent mono- or polysynaptic inputs from the BLA and VTA.

Experiments

Electrophysiological experiments in freely moving rats during free drinking of sugar solution

Methods

Animals

Three male Wistar rats (400-500g) were used. Animals were handled daily and were kept at 12-12 h dark/light cycle in a temperature controlled vivarium. Prior to electrode implantation standard rat food (CRLT/N, Charles River Laboratories, Budapest, Hungary) and tap water were available at lib. In order to motivate water intake during experiments water was only available during experimental sessions and 20 min period after experiments in the housing cage. Body weight was kept at 80-90% of the normal value.

Electrode implantation

Prior to experiments rats were implanted with printed circuit board based microdrives loaded with 8 tetrodes (Toth et al., 2007; Petyko et al., 2009) (Bregma AP: 2,7-3,7; DV: 3,2-4,4; ML: 0,5-0,8 according to the atlas from Paxinos and Watson) (Paxinos and Watson, 1996). During surgery rats were anesthetized with Na-pentobarbital (Nembutal, 60 mg/kg, Phylaxia-Sanofi, Hungary).

Behavioral procedure and equipment

Experiments started after 2 weeks recovery in a 40 x 40 cm operant box. Between experimental sessions rats were not allowed to drink, but food was available ad lib. During experimental sessions rats were first allowed to drink water until satiation (17-34 min; 13-27 ml). Then the drinking bottle was changed to another one containing 5% sucrose solution. Rats drank this solution until satiation (60min; 23-35 ml). Behavior of animals was recorded with a webcam using the Spike2 software (10 frame/sec). An important unit of the licking structure of rat is the licking cluster. Licking clusters are defined by an at least 0,5 sec pause before the first lick (Davis and Smith, 1992). Beginnings of licking clusters were determined by the analysis of the recorded videos (0.1 sec resolution).

Electrophysiological recordings

The recording system was regularly tested with a 32-channel signal generator (Mathe et al., 2007). For recording of electrophysiological data a 32-channel head stage amplifier was (Noted Bt., Hungary) connected to the interface board on the microdrive (Toth et al., 2007). The 8 tetrodes were moved if necessary in 150 μ m steps after experimental sessions. Each movement of the tetrodes was noted. The outputs of the preamplifier was connected via tether cable to the tetrode selector (Noted Bt., Hungary). The 2 tetrodes with the best signal quality were chosen at the beginning of experimental sessions. This 8 channels (2 tetrodes) were connected to an Axon Cyberamp 380 amplifier, filtered (300-6000 Hz) and amplified. The amplified signals were digitized at 41 kHz with a CED micro interface (Cambridge Electronic Designs). Data were visualized, recorded on a PC and analyzed with the Spike2 software.

Analysis of electrophysiological data and histology

During offline analysis raw electrophysiological data were digitally filtered (700-3000 Hz), with the principal component analysis and cluster analysis function of the Spike2 software, spike-s were sorted into clusters. Activity changes were detected by using perievent histograms (PETH). For detecting significant activity changes a Poisson-test for PETHs was applied (Dorrscheidt, 1981). Electrode tracks were detected with toluidin-blue staining of the brain slices. Recording sites were reconstructed according electrode movements.

Results

Neuronal responses

Out of the 76 tested neurons 38 changed their activity significantly in relation to sugar solution (99% confidence interval). The majority of these neurons (33 out of 38) only changed their activity significantly in relation to water solution. Only 5 neurons changed their activity time locked to licking clusters independently from the reward (sugar solution or water).

Neuronal responses time locked to and just before licking clusters

More the half of responding neurons (n=20) changed their activity just before licking clusters ("before licking cluster (BL) neurons").

Excitatory "before licking cluster" neurons

The majority of the above mentioned 20 neurons (n=16) changed their activity maximally during the 0.2-1.0 s interval before licking cluster onsets (excitatory BL neurons). Three of these neurons also responded to drinking of water, but there was no significant difference between responses in relation to sugar solution or water. There was a characteristic response pattern for all excitatory BL neurons. The onset of activation was 0.6-1 sec before and the maximal activation was 0.6-0.3 sec before licking cluster onset. The activity of neurons returned to baseline level during the 0.2 sec interval just before the licking clusters.

Inhibitory "before licking cluster" neurons

A smaller number of BL neurons was maximally inhibited just before licking cluster onsets (inhibitory BL neurons). The time course of responses was similar to those of excitatory BL neurons. These neurons were maximally inhibited 1.2 sec before licking cluster onsets. None of these neurons responded in relation to water.

Neurons responding during licking clusters

Another remarkable group of neurons (n=18) changed the firing frequency during licking clusters ("licking cluster (LC) neurons").

Excitatory "licking cluster" neurons

The majority of these neurons (n=11) increased their firing frequency 0.3-1.2 sec after licking cluster onsets (excitatory LC neurons). Only one of these neurons also responded to water but there was no significant difference between responses to different rewards. There was a characteristic response pattern for all excitatory LC neurons, an elevation of firing frequency was observed 0.3-1.2 sec after licking cluster onsets. In contrast to BL excitatory neurons the decrease of responses was variable. The activity of most of these neurons returned to baseline level within 2 sec. Two neurons showed elevated firing frequency until the end of licking clusters.

Inhibitory "licking cluster" neurons

A smaller number of LC neurons (n=7) decreased the firing frequency during licking clusters (inhibitory LC neurons). Similarly to excitatory neurons the beginnings of responses were constant and the ends of responses were variable. Only one inhibitory LC neuron changed its activity significantly to water. This neuron reached significant firing frequency decrease late in the 4th second of the licking cluster.

Electrophysiological experiments on freely moving rats during modified Pavlovian trace conditioning

Questions

In order to study the role of the mPFC in reward prediction, as a consequence of stimulus-reward association, we investigated the possible representations of liquid food reward in responses of neurons: (i) during different CSs; (ii) during approach behavior as CR; (iii) during US elicited licking behavior (consummation); (iv) and the possible representation of consummation and CR in neurons which also respond to CSs.

Methods

Animals

The three rats (400-500 g) were cared as described in the previous experiment.

Electrode implantation

Electrodes were implanted as described in the previous experiment.

Behavioral procedure and apparatus

Experiments were performed in a 40 x 40 cm sound attenuated box in the light period. For delivery of sugar solution or water two drinking bottles were used which could be moved by a computer controlled solenoid. Experimental sessions began after 23 h water deprivation. The two different rewards (5% sugar solution or water) were paired with two different tones, the sugar solution with 16 kHz tone (CS1-US1) and water with a 8 kHz tone (CS2-US2). A fix interstimulus interval (24 sec) was used. The 2 sec CS was followed by a 1 sec trace interval (trace conditioning). This was followed by the US (12 sec). During the experimental session 50-50 CS1-US1 and CS2-US2 pairings were presented pseudorandomly. After conditioning data from totally 24 experimental sessions were analyzed.

Lickings were detected by a small current between the rats tongue and drinking tube (100 nA). CS, US and licking cluster onsets were recorded to the electrophysiological data with ms resolution.

Recording of data and spike sorting

For electrophysiological recording of neuronal activity and head acceleration, a miniature 32-channel head stage amplifier with built-in accelerometer (Noted Bt., Pécs, Hungary) was plugged into the socket at the interface board on the microdrive. Wide-band signals (0.1 Hz-18.75 kHz) from 8 tetrodes (32 channels), and events were recorded

continuously by means of a 24-bit, 64-channel low voltage AD converter (LVC-64, Noted Bt., Pécs, Hungary). The recorded wide-band signals were digitally high pass filtered (0.8–5 kHz). Spikes were extracted after automatic threshold detection (Csicsvari et al., 1998). Employing principal component analysis 12-dimensional feature vector was created for each spike (first three principal components for each channel from a tetrode) (Csicsvari et al., 1998). Spikes from putative individual neurons were segregated using automatic clustering software KlustaKwik (Harris et al., 2000). The generated clusters were tested and refined by a graphical clustering software (Klusters) (Hazan et al., 2006).

Data analysis

Off-line analysis of data, generation of raster plots and perievent time histograms (PETH), and the statistical analysis were performed using Matlab and shell scripts written in Linux.

Peri-event time histograms (PETH) were used to detect single neuron responses time-locked to events related to CSs and licking cluster onsets. The onset of a licking cluster was defined by the first lick after an interlick interval of more than 0.5 sec (Davis and Smith, 1992). CS onsets were used to detect neuronal activity changes in relation to associated reward. Neuronal responses before and time locked to licking cluster onsets were analyzed to detect neuronal responses related to CS elicited approach behavior as conditioned responses. Neuronal responding during licking clusters was also analyzed to detect activity changes related to consummation of appetitive reward.

PETHs were normalized, and for each bin the z-score was calculated from an approximate Poisson distribution of the expected firing rate. If the z-score was ≥ 2.36 or ≤ -2.36 in at least 3 consecutive bins, the neuron was considered as responding to the event ($p < 0.01$) (Totah et al., 2009). For the analysis of population activity, the z-scores of each responding unit were averaged and ANOVA was performed. Whenever indicated by ANOVAs, Tukey's HSD test was used for post-hoc statistics ($p < 0.05$).

Before the licking clusters rats approached the drinking tube with a fast stereotype movement. This movement was a potential CR and was measured with an accelerometer mounted to the head. The maximal head acceleration was calculated and analyzed with one-way ANOVA ($p < 0.01$). Tukey HSD test was applied in order to detect significant differences between the different situations ($p < 0.05$).

Histology

After finishing the experiments, transcardial perfusion was performed, followed by the removal of the brain from the skull. Brains were cut into sections of 40 micrometer thickness, and toluidine-blue histological staining was performed to detect electrode tracks. Electrode tracks were reconstructed after microscopic evaluation and the recording sites were identified considering the electrode positions.

Results

Histology

All recording sites were localized in the layers III-VI of the prelimbic area and the lower border of the anterior cingulate cortex area (from Bregma, AP: 2.2–3.2; DV: 3.2–4.4; ML: 0.45–0.11)

Head acceleration during approach behavior

Approach behavior was quantified by measuring the head acceleration just before the first licks of licking clusters. The first licks occurred usually during the first 2 sec of US delivery (early approach). Later occurring approaches were subdivided into late occurring first approaches (late approach) and approaches occurring between licking clusters (intercluster approach). The combination of these events with the US types (sugar or water) resulted in a total of 6 distinct values of the factor situation. A one-factor ANOVA revealed significant main effect due to factor situation ($F(5,417)=48.72$, $p<0.0001$). The Tukey's HSD test was performed to compare the 6 different situations. There was a significant difference ($p<0.05$) between the two US types when comparing the early approaches. Compared to the water reward situation, clearly higher accelerations were observed during approaches to the drinking bottle containing sugar solution. The lower accelerations were observed during intercluster approaches when there was no significant difference between the US types. In case of sugar solution reward, the acceleration proved to be significantly different in the early approach situation compared to both late situations. A similar difference could be observed in the case of water.

Neuronal responses

Hundred and twenty neurons of 211 neurons (56,9 %) responded to at least one event. ($|z|>2.36$; $p<0.01$). Many neurons responded to different events.

Neuronal responses during the CSs

Among all studied neurons, 41 (19,4%) changed their firing rate to reward predicting tones. Neuronal activities during the CSs were analyzed as two different trial types (consummation and no consummation) for sucrose solution and two different trial types for water (totally 4 trial types).

Single unit responses

Twenty seven neurons out of 211 (12,8%) increased their firing rate during cue tones predicting sugar solution or water. Out of these 27 neurons, 24 responded to CS only during trials when the rats drank the predicted solution. Sixteen cells responded to both cue tones, whereas 8 neurons only to the tone predicting sugar solution and 3 only to the tone predicting water. Out of the 27 neurons, 12 exhibited various activity changes during licking clusters.

Fewer neurons, altogether 14, decreased their firing rate during cue tones predicting sugar solution or water. Out of these 14 neurons, 11 responded to CS only during trials when the rats drank the predicted solution. Seven neurons changed in activity to both cue tones, 2 responded only to the tone predicting sugar solution, and 5 only to the tone predicting water. Six out of the 14 neurons exhibited various responses during licking clusters.

Population activity

We calculated the population activity of the 27 neurons exhibiting excitatory responses to at least one of the CSs. A clear steep increase in the average of z-values could be seen after acoustic cue onsets, during trial types when rats drank the sugar solution or water. The population activity was higher for the tone predicting sugar solution. The population activities during trials when the rats drank the rewards were clearly higher than those during trials when the rats did not drink the reward, in the latter case the activities barely exceeded the baseline level. There was a significant main effect due to factor time ($F(19,2080)=17.19$, $p<0.0001$) and trial type ($F(3,2080)=73.66$, $p<0.0001$). There was also a significant interaction between trial type and time ($F(57,2080)=4.36$, $p<0.0001$). The Tukey's HSD test revealed significant difference ($p<0.05$) between trial types when the rats drank the sugar solution or water and when they did not drink the reward solutions. There was also a significant difference between population responses to the two different CS tones when rats drank the predicted solutions as reward.

We calculated the population activity of the 14 neurons exhibiting inhibitory responses to at least one of the CSs. In contrast to excitatory units, the population activity of inhibitory cells decreases continuously beginning 2 sec before cue onset during trials when the rats drank the rewards. There was no difference between trials when the rats drank sugar solution or water. The population activity during trials when the rats did not drink the reward was not distinguishable from the baseline level. For the inhibitory units, a significant main effect was observed due to factor time ($F(19,1040)=14.77$, $p<0.0001$) and trial type ($F(3,1040)=63.17$, $p<0.0001$). There was also a significant interaction between trial type and time ($F(57,1040)=3.95$, $p<0.0001$). The Tukey's HSD test revealed significant difference ($p<0.05$) between trial types when the rats drank the sugar solution or not and when the rats drank water or not. In contrast to the excitatory responses, there was no significant difference between trial types when the tones predicted sugar solution or water and the rats drank them as reward.

Neuronal responses just before and time locked to licking cluster onsets

Among all studied neurons (211), 56 (26.5%) neurons changed their firing rate before the first lick of licking clusters.

Single unit responses

Thirty (14.2%) out of the 211 neurons exhibited excitatory responses just before licking clusters. Out of the 30 neurons, 12 responded before both rewards, 13 only before licking clusters when the rats drank sugar solution, and 5 before licking clusters when the rats drank water. Fourteen out of the 30 neurons exhibited responses of yet unknown significance during licking clusters, too.

A similar proportion, 26 out of 211 (12.3%) neurons, decreased their firing rate just before licking clusters. Out of the 26 neurons, 15 responded before both rewards, 8 responded only to the tone predicting sugar solution, whereas 3 responded only to the tone predicting water. The overwhelming majority, 22 out of the 26 neurons, exhibited - mostly inhibitory - responses (20 inhibitory; 2 excitatory) during licking clusters.

Population activity

We calculated the population activity of the 30 neurons exhibiting excitatory responses before the first lick of licking clusters. The population response was higher before the first lick of licking clusters when the rats drank sugar solution. There was a significant main effect due to factor time ($F(19,1160)=22.43$, $p<0.0001$) and trial type ($F(1,1160)=28.25$, $p<0.0001$).

There was also a significant interaction between trial type and time ($F(19,1160)=1.96$, $p<0.01$).

In order to find out the relation of these responses to head acceleration, we subdivided the first licks of licking cluster according to the six situations used to analyze head accelerations. Only a small subset of neurons could be included in this analysis, and no significant difference was revealed in the Tukey's HSD test. In a separate analysis, it was tested whether the excitatory population activity when the first lick took place during the first 2 sec of US delivery (early approach) differed from the population activity taking place in later phase independently from whether it was defined as late or intercluster approach. The combination of these phases with the US type (sugar or water) resulted in a total of 4 values of the factor situation. There was a significant main effect due to factor time ($F(19,2320)=38.16$, $p<0.0001$) and due to factor situation ($F(3,2320)=9.86$, $p<0.0001$). The Tukey's HSD test was performed to compare the 4 different situations (Figure 6). Comparing the two early situations, there was a significant difference ($p<0.05$) between the two US types. Similarly, when comparing the late/intercluster situations, also there was a significant difference ($p<0.05$) between the two US types. In contrast to the behavioral results, there was no significant difference observed between the early and late/intercluster situations when the rats drank sugar solution. Similarly, there was no significant difference between the early and late/intercluster situations when the rats drank water.

We calculated the population activity of the 26 neurons exhibiting inhibitory responses before the first lick of licking clusters. There was a significant main effect due to factor time ($F(19,1000)=22.43$, $p<0.0001$), but there was no significant effect due to trial type, and there was no significant interaction as well.

Neurons responding during licking clusters

A significant proportion, 80 (37.9%) out 211 neurons changed their firing rate after the first lick of licking clusters.

Single unit responses

Out of the 80 neurons, 33 (15.6%) exhibited excitatory responses. Out of these 33 neurons, 14 responded to both kinds of rewards, 9 only during licking clusters when the rats drank sugar solution, and 10 during licking clusters when the rats drank water.

Altogether 47 neurons (22.3%) displayed inhibitory activity changes. Out of these 47 neurons, 28 responded to both types of rewards, 12 only during licking clusters when the rats drank sugar solution, and 7 during licking clusters when the rats drank water.

Population activity

We calculated the population activity of the 33 neurons exhibiting excitatory responses after the first lick of licking clusters. The population response was higher after the first lick of licking clusters when rats drank sugar solution. There was a significant main effect due to factor time ($F(34,2240)=8.81$, $p<0.0001$) and trial type ($F(1,2240)=31.82$, $p<0.0001$), respectively. There was, however, no significant interaction between trial type and time.

We calculated the population activity of the 47 neurons exhibiting inhibitory responses after the first lick of licking clusters. There was a significant main effect due to factor time ($F(34,3220)=50.53$, $p<0.0001$) and trial type ($F(1,3220)=71.26$, $p<0.0001$), but there was no significant interaction.

Neurons modulated by licking rhythm

Single neuron activity

Nineteen (9%) out of the 211 neurons displayed modulation of firing rate around the lick onset. Twelve neurons displayed firing rate increase just after drinking tube contact, 7 neurons displayed firing rate decrease. Two of 19 neurons also displayed significant firing rate changes during cue tones, 2 before the first licks of licking clusters and 3 during the licking clusters.

Juxtacellular electrophysiological experiments on anesthetized rats

The relation between electrophysiological single unit response types in the mPFC elicited by electrical stimulation of the BLA and VTA and morphological neuron types is less understood.

In the experiment presented here the BLA and VTA were stimulated, and juxtacellular single unit recordings were performed in the mPFC. After electrophysiological characterization neurons were filled juxtacellularly with Neurobiotine (Pinault, 1994). The aim of this experiment was the Golgi-like labelling of mPFC neurons responding to BLA and/or VTA stimulation and to examine the relation between electrophysiological response types (Perez-Jaranay és Vives, 1991; Piroot és mts., 1992) and neuron types described in Golgi-studies (Vogt and Peters, 1981).

Methods

Animals and surgery

Eighty-three male Wistar rats (350-400 g) were used. They were anesthetized with Urethane (1.2 g/kg), fixed in the stereotaxic apparatus and operated on. Body temperature was kept at 36.5-37.5 °C with a water heating system. Holes were made in the skull for electrode placement. The following stereotaxic coordinates were used (Paxinos and Watson, 1996) BLA stimulation: Bregma AP: -2,5; DV: 8; ML: 5; VTA stimulation: Bregma AP: -6,04; DV: 7,4; ML: 0,5; recording in the mPFC: Bregma AP: 2,7-3,7; DV: 0,5-3; ML: 0,3-1,5.

Electric stimulation of the BLA and VTA

Concentric bipolar stimulating electrodes (diameter 0.2 mm; tip-ring distance 0.5 mm) were moved into the BLA and VTA with mechanical micromanipulators (Narishige Japan). Monophasic square impulses were generated with a Grass S88 stimulator and PSIU6 (Grass) stimulus isolation unit.

Juxtacellular recording a labeling of neurons with Neurobiotine

Glass electrodes (borosilicate glass; tip 1-2 µm; 2% Neurobiotine dissolved in 0.5 M NaCl) were used for extracellular recordings. They were moved in the brain by means of a mechanical micromanipulator (Eric Sobotka). Action potentials were amplified with the "iontophoretic preamplifier" and main amplifier and filtered (2000x; 300-5000 Hz). The

signals were also monitored by a Tektronix TDS 210 oscilloscope. Data were recorded with a computer using a CED 1401 plus interface (Cambridge Electronic Design Ltd.).

Data analysis

Action potentials were sorted offline with cluster analysis using the Spike2 software. Effects of BLA and VTA stimulations were detected on PSTHs (bin size: 1ms; bin number: 400; offset: 100ms). Onsets of significant activity changes were determined with a cumulative sum test (Cusum). After electrophysiological characterization neurons were labeled with Neurobiotine (Pinault, 1994). A low noise current generator was connected to the input of an AC preamplifier (Supertech, Hungary). Correlations between rates of morphological and electrophysiological types were analyzed with the Fischer exact test (Stat 100 software).

Neurobiotine histochemistry

One or two hours after Neurobiotine labeling rats were transcardially perfused with physiological saline, after this with 4% paraformaldehyde solution in phosphate buffer (PB). Brains were removed from the skull and kept for 20 h in PB containing 30% sucrose. Brains were cut with a freezing microtome (40 μm coronal sections), and the Neurobiotine was visualized with ABC histochemistry (Pinault, 1994). The nickel-DAB product was silver-intensified (Merchenthaler et al., 1989). The coronal sections were mounted on slides and covered with Pertex. Labeled mPFC neurons were drawn by using a camera lucida (Nikon) and photographed (Nikon Optiphot 2).

Results

Single-unit responses

Single-unit responses were recorded from 82 mPFC neurons. The spontaneous activity of neurons varied between 0.2 Hz and 24 Hz (half of neurons between 1 and 6 Hz). 55 neurons responded with inhibition, 6 with excitation, 21 did not respond.

In case of totally 30 neurons responding to BLA stimulation the effect of VTA stimulation was also investigated. Out of 23 neurons also responding to VTA stimulation 2 responded with excitation to BLA stimulation and 21 with inhibition. Two neurons which were inhibited by both VTA and BLA stimulation showed antidromic excitation by high intensity VTA stimulation.

Morphology of neurons labeled with Neurobiotine

We only tried to fill the responding neurons. Out of the 55 responding neurons in 36 cases was the spontaneous activity modulated by iontophoretical application of Neurobiotine. Brain slices were processed for Neurobiotine histochemistry. In 31 out of the 36 rats only 1 labeled neuron could be seen at the end of the electrode track. If the end of the electrode track was visible on the slide, it was located in a juxtacellular position.

Labeled neurons were classified according Golgi studies (Vogt and Peters, 1981): 1) small and medium sized pyramidal cells: perikarya less pyramidal shaped, size 8-12 μm , poor dendritic arborization; large pyramidal cells: perikarya 12-20 μm , extensive dendritic arborisation; 4) small and medium sized multipolar neurons: perikarya 8-12 μm .

Responses of morphologically characterized neurons to BLA/VTA stimulation

21 neurons were successfully labeled in the dorsal mPFC. All small to medium sized pyramidal cells (16) responded with short latency (20-25 ms) inhibition to BLA stimulation.

The 4 large pyramidal cells showed excitatory responses to BLA stimulation followed by a silent period, 1 showed short latency (20-25 ms) and 1 showed long latency (55 ms) response.

There was a significant difference in relation to the 20-25 ms latency inhibition elicited by BLA stimulation. All small to medium sized pyramidal cells showed short latency inhibition, contrary to this only 2 of 4 large pyramidal cells responded similarly (Fischer Exact test; $p=0,0035$). There was also a significant difference in relation to excitatory responses (only large pyramidal cells) (Fischer Exact test; $p=0,0316$).

The labeled multipolar interneuron exhibited (presumably monosynaptic) excitatory responses followed by a silent period.

Out of the 10 labeled pyramidal cells responding to BLA stimulation also responded (with inhibition) to VTA stimulation. Nine of these neurons responded with inhibition to BLA stimulation 1 with excitation. Two pyramidal cells (1 large, 1 small) (inhibited by BLA and VTA stimulation) exhibited antidromic excitation by VTA stimulation.

Discussion

Significance of licking clusters

Liquid food is ingested by rat through licking behavior, which is a complex motor action. It is organized into bursts of licks. Within the licking burst the frequency is constant and controlled by a central rhythm generator. Bursts are interrupted by side-movements of the tongue leading to 250-500 ms interburst intervals. One or more bursts occur in clusters that are separated by intervals that range from 500 ms to seconds (Davis and Smith, 1992; Gutierrez et al., 2006). Licking clusters represent motivated behavior (Gutierrez et al., 2006): the size and number of clusters alter with the palatability of taste and they are correlated with the development of satiety. In our first two experiments, we studied the neuronal activity of the mPFC in relation to licking clusters. Because neuronal responses occurred typically around the cluster onsets, we analyzed in details the single neuron activity changes around licking cluster onsets.

Significance of sugar solution

The mPFC is connected with several brain structures involved in reward-related mechanisms: the OBF, ventral tegmental area, lateral hypothalamus, and Acb (Beckstead, 1979). Disruption of the bidirectional intercortical connection between the OBF and the mPFC impairs the association between the taste and the place (Schalomon et al., 1994). Moreover, in the mPFC, the extracellular dopamine concentration is increased in case of feeding of novel or palatable foods (Bassareo and Di Chiara, 1999). According to the above facts, we have hypothesized that neuronal firing rate changes may occur in the mPFC during ingestion of palatable foods. Therefore we studied responses that occur during licking of a rewarding liquid food.

Neurons responding during licking clusters

In our first 2 experiments a significant proportion of neurons (23% and 38%) changed their firing frequency during licking clusters, in both experiment excitatory and inhibitory responses were observed. **We demonstrated that responses are not related to drinking as a motor process**, because there is no difference in drinking during the licking clusters (e.g. the same licking frequency) but the neuronal responses are different. In a recent paper (Horst and Laubach, 2013) the onset of licking behavior could be separated from reward delivery. In our second experiment we could not separate licking from consumption, Despite of these fact we assume that both, licking behavior and consumption contribute to single unit responses observed during licking clusters. **We assume that both taste and reward value are important for the observed responses**. In agreement with this, in an another paper it was shown, that mPFC neurons code sensory properties of the food, specifically the taste or palatability or both (Jezzini et al., 2013). Totally 19 neurons modulated their firing frequency corresponding to licking rhythm. Surprisingly only 3 neurons responding during licking clusters did so. We concluded that **responses during licking clusters are not a consequence of the modulation by licking rhythm**. Interestingly a few neurons, in contrast to the majority did not differentiate between sugar solution and water, the responses of these neurons could be related to the consumption itself (Horst and Laubach, 2013).

Neurons responding just before licking clusters

Single unit responses related to reward expectancy were described in the monkey dorsolateral PFC (Watanabe, 1996) in a brain region which corresponds at least partly to the rat mPFC (Gabbott et al., 2003). In the first 2 experiments presented here 26% of neurons changed the firing frequency just before licking clusters. We assumed that these are related to reward expectancy, but we could not prove this in our first experiment. In Pavlovian situation the approach of the drinking tube was a CS dependent Pavlovian CR, correspondingly rats approached the drinking tube faster (higher head acceleration). The fact, that population activity of neurons was independent of head acceleration (similar responses during early and late/intercluster approach) evidenced our hypothesis, that **majority of single unit responses are not related to approach behavior, but it is related to reward expectancy**. Indeed at single cell level many neurons exhibited response patterns independent of head acceleration. But some neurons modulated their firing frequency correspondingly to head acceleration, **the responses of these neurons was related to approach behavior**. Single unit responses related to jaw movements (CR) were detected in the rabbit mPFC (McLaughlin et al., 2002). These neurons respond to the CS+ but do not respond to the CS-. Our results are in agreement with the cited paper, because during the early phase the population activity was different between the different trial (reward) types, during which different head accelerations were observed.

Neuronal activity changes during CSs predicting different rewards

In the Pavlovian situation about 1/5 of neurons responded to CSs predicting different rewards. **A striking result was, that majority of neurons responded to the CS only during trials with consummation of the reward**. Neurons did not respond when rats switched to non-consummatory behavior patterns (rearing, walking, scratching, exploration, resting), thus **responses to CSs are strongly related to the Pavlovian situation itself**. We can also conclude, **that most responses are not related to the sensory properties of the CS**. This statement is also supported by the observation, that some neurons exhibited similar responses

to both CSs. Other neuron groups exhibit higher responses to the CS+ or CS-. Neuronal responses related to Pavlovian appetitive conditioning have been described in the rabbit mPFC (McLaughlin et al., 2002). Neurons have been detected which responded to the CS+ associated with reward, but did not respond to the CS- which was not associated with reward. These results and our result suggest, that responses of mPFC neurons during CS tones code stimulus-reward association. "Bridging neurons" have been detected during trace conditioning in aversive situation (Siegel et al., 2012; Gilmartin et al., 2014). The sustained activity of these neurons (coding information about the CS) during the trace interval is necessary for CS-US association. The 1 sec trace interval during our experiment was too short to analyze bridging activity. Despite of this, **some neurons maintained their increased activity after the CS**, this neurons are likely "bridging neurons". We hypothesize, that **an important function of neurons during the CS is related to bridging of the trace interval**. It is largely accepted, that a function of mPFC neurons is the inhibition of behavior (Ongur and Price, 2000). In agreement with this a striking result in our experiments was, that during non-consummatory behavior neurons did not respond to CSs and did not show any activity related to behavior, many neurons also decreased their spontaneous activity. It is possible, that in this situation the inhibition of the mentioned non-consummatory behaviors stops, but during Pavlovian conditioning these behaviors are inhibited.

Single unit responses to BLA and VTA stimulation in the rat mPFC

Single unit responses to BLA and VTA stimulation were recorded in the mPFC of anesthetized rats. **About two third of mPFC neurons responded with inhibition to BLA stimulation, a smaller proportion responded with excitation**, about one third did not respond. Response types and spontaneous firing frequencies was in agreement with previous papers (Perez-Jaranay és Vives, 1991; Pirot és mts., 1992; Floresco és Tse, 2007).

Morphology of mPFC neurons

The morphology of mPFC neurons corresponded to the results of Golgi studies published earlier (Vogt and Peters, 1981). However, when results were compared to immunohistochemical studies (Gabbott et al., 1997) we could not detect different interneuron types. There are several methodological reasons for this discrepancy. After electrophysiological characterization only one neuron was labeled, this was only in case of half of neurons possible, so the total number of neurons was low. Besides this, it is possible that the juxtacellular method is selective for particular neuron types.

Responses of "small to medium sized pyramidal cells" to BLA/VTA stimulation

Totally 22 electrophysiologically characterized neurons were also morphologically characterized. **16 neurons were "small to medium sized pyramidal cells", all of these 16 neurons responded with short latency (20-25 ms) inhibition to BLA stimulation**. According to previous and our results the 20-25 ms latency inhibition is the most frequent response to BLA stimulation (Perez-Jaranay and Vives, 1991). But the high frequency of such responses found in our experiment differs significantly from a random distribution (Fischer Exakt teszt; $p=0,0035$). 8 of these 16 neurons also responded to VTA stimulation with inhibition and 1 exhibited antidromic excitation to high intensity VTA stimulation, so the mPFC neuron projected back to the region in the VTA from which it received inhibitory input.

Responses of "large pyramidal cells" to BLA/VTA stimulation

Out of 4 "large pyramidal cells" 2 exhibited 7 ms onset latency excitatory responses (monosynaptic), 1 20-25 ms inhibitory and 1 long latency (polysynaptic) inhibitory responses (Beckstead, 1979; Perez-Jaranay and Vives, 1991; Gabbott et al., 1997). This result suggests, that **"large pyramidal cells" differ functionally from "small to medium sized pyramidal cells" with regard to their input from the BLA.** One "large pyramidal cell" exhibiting excitatory and another one exhibiting long latency inhibition showed inhibitory responses to VTA stimulation. The last mentioned neuron was antidromically excited by VTA stimulation, so the neuron projected back to the region in the VTA from which it receives inhibitory input.

Responses of "large multipolar cells" to BLA/VTA

The "large multipolar cell" which belongs to interneurons exhibited 7 ms onset latency (monosynaptic) excitatory responses according to the literature (Gabbott et al., 2006; Floresco and Tse, 2007). The neuron exhibited inhibitory responses to VTA stimulation.

The connections of the mPFC (aCg/PL) with the BLA and VTA and its role in motivation

The BLA sends a monosynaptic glutamatergic input to interneurons and pyramidal cells in the mPFC. But parvalbumine-positive GABA-ergic interneurons inhibit monosynaptically many pyramidal cells (Gabbott et al., 2006). So electric stimulation of the BLA causes monosynaptic excitation and bisynaptic inhibition of many pyramidal cells. **A new result in our experiment was, that in case of BLA stimulation on "small to medium size pyramidal cells" dominates the bisynaptic inhibition (20-25 ms onset latency). Contrary, in case of "large pyramidal cells" we found monosynaptic excitation, bisynaptic inhibition and polysynaptic inhibition.** The last one occurred likely via the mediodorsal thalamic nucleus, glutamatergic inputs from here innervate pyramidal cells and inhibitory interneurons. (Beckstead, 1979; Perez-Jaranay and Vives, 1991; Gabbott et al., 1997). Dopaminergic inputs from the VTA terminate directly on pyramidal cells and presynaptically on fibers arising from the thalamus and hippocampus. Stimulation of the VTA leads usually to massive inhibition of mPFC neurons. **A new result in our experiments was, that some "small to medium sized pyramidal cells" and "large pyramidal cells" receive convergent inputs from the BLA and VTA and project back to the VTA (antidromic excitatory response).**

The mPFC, the BLA and the VTA play a role in several aspects of the motivated behavior. In BLA lesioned animals the instrumental response to a Pavlovian CS serving as secondary reinforcer is decreased (Cador et al., 1989). The BLA plays a role in relation to the incentive value of rewards (Malkova et al., 1997). Closely related to this it is necessary for conditioned taste aversion (CTA) learning and retention (Yamamoto and Ueji, 2011). Disturbance of CTA was also detected after kainate and 6-OHDA lesions of the mPFC (Hernádi és mts., 2000; Karádi és mts., 2005). Since both BLA and mPFC are important for CTA, the connections between them, which mediate the electrical stimulation may also affect CTA. On the other hand the function of the VTA is related to the incentive motivational value of stimuli related to rewards (Berridge and Robinson, 1998).

In case of electric stimulation of anesthetized rats one can only speculate which behavior might be affected by the investigated neurons. Besides, the response patterns elicited by electric stimulation differs from physiological ones. Nevertheless we can conclude, that information from brain regions involved in motivation is integrated in complex manner in the

mPFC. According to our results "small to medium sized" and "large pyramidal cells" may belong to functionally distinct parallel systems.

References

- Balleine BW, Dickinson A (1998) Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. *Neuropharmacology* 37:407-419.
- Bassareo V, Di Chiara G (1999) Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. *Eur J Neurosci* 11:4389-4397.
- Beckstead RM (1979) An autoradiographic examination of corticocortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. *J Comp Neurol* 184:43-62.
- Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev* 28:309-369.
- Cador M, Robbins TW, Everitt BJ (1989) Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. *Neuroscience* 30:77-86.
- Coutureau E, Dix SL, Killcross AS (2000) Involvement of the medial prefrontal cortex-basolateral amygdala pathway in fear-related behaviour in rats. *Eur J Neurosci* 12(Suppl. 11):156.
- Csicsvari J, Hirase H, Czurko A, Buzsaki G (1998) Reliability and state dependence of pyramidal cell-interneuron synapses in the hippocampus: an ensemble approach in the behaving rat. *Neuron* 21:179-189.
- Davis JD, Smith GP (1992) Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. *Behav Neurosci* 106:217-228.
- Dickinson A (1994) Instrumental conditioning. In: *Animal learning and cognition* (Mackintosh NJ, ed), pp 45-79. San Diego: Academic Press.
- Dorrscheidt GH (1981) The statistical significance of the peristimulus time histogram (PSTH). *Brain Res* 220:397-401.
- Floresco SB, Tse MT (2007) Dopaminergic regulation of inhibitory and excitatory transmission in the basolateral amygdala-prefrontal cortical pathway. *J Neurosci* 27:2045-2057.
- Gabbott PL, Warner TA, Busby SJ (2006) Amygdala input monosynaptically innervates parvalbumin immunoreactive local circuit neurons in rat medial prefrontal cortex. *Neuroscience* 139:1039-1048.
- Gabbott PL, Warner TA, Jays PR, Bacon SJ (2003) Areal and synaptic interconnectivity of prelimbic (area 32), infralimbic (area 25) and insular cortices in the rat. *Brain Res* 993:59-71.
- Gabbott PL, Dickie BG, Vaid RR, Headlam AJ, Bacon SJ (1997) Local-circuit neurones in the medial prefrontal cortex (areas 25, 32 and 24b) in the rat: morphology and quantitative distribution. *J Comp Neurol* 377:465-499.
- Gilmartin MR, Balderston NL, Helmstetter FJ (2014) Prefrontal cortical regulation of fear learning. *Trends Neurosci* 37:455-464.
- Grastyan E (1976) Motivation and reinforcement. *Acta Physiol Acad Sci Hung* 48 (4):299-322.
- Grastyan E, Karmos G, Vereczkey L, Martin J, Kellenyi L (1965) Hypothalamic Motivational Processes as Reflected by their Hippocampal Electrical Correlates. *Science* 149:91-93.

- Gutierrez R, Carmena JM, Nicolelis MA, Simon SA (2006) Orbitofrontal ensemble activity monitors licking and distinguishes among natural rewards. *J Neurophysiol* 95:119-133.
- Hall J, Parkinson JA, Connor TM, Dickinson A, Everitt BJ (2001) Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating Pavlovian influences on instrumental behaviour. *Eur J Neurosci* 13:1984-1992.
- Harris KD, Henze DA, Csicsvari J, Hirase H, Buzsaki G (2000) Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements. *J Neurophysiol* 84:401-414.
- Hatfield T, Han JS, Conley M, Gallagher M, Holland P (1996) Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. *J Neurosci* 16:5256-5265.
- Hazan L, Zugaro M, Buzsaki G (2006) Klusters, NeuroScope, NDManager: a free software suite for neurophysiological data processing and visualization. *J Neurosci Methods* 155:207-216.
- Hebb DO (1955) Drives and the C.N.S. (conceptual nervous system). *Psychol Rev* 62:243-254.
- Hoebel BG, Monaco AP, Hernandez L, Aulisi EF, Stanley BG, Lenard L (1983) Self-injection of amphetamine directly into the brain. *Psychopharmacology (Berl)* 81:158-163.
- Horst NK, Laubach M (2013) Reward-related activity in the medial prefrontal cortex is driven by consumption. *Front Neurosci* 7:56.
- Hull CL (1943) *Principles of behavior*. New York: Appleton.
- Jezzini A, Mazzucato L, La Camera G, Fontanini A (2013) Processing of hedonic and chemosensory features of taste in medial prefrontal and insular networks. *J Neurosci* 33:18966-18978.
- Kluver H, Bucy PC (1939) Preliminary analysis of functions of the temporal lobes in monkeys. *Arch Neurol Psychiatry* 42:979-997.
- LeDoux JE (2000) The amygdala and emotion: a view through fear. In: *The amygdala: a functional analysis*, 2 Edition (Aggleton JP, ed), pp 289-310. New York: Oxford University Press.
- Lenard L, Hahn Z (1982) Amygdalar noradrenergic and dopaminergic mechanisms in the regulation of hunger and thirst-motivated behavior. *Brain Res* 233:115-132.
- Lenard L, Hahn Z, Karadi Z (1982) Body weight changes after neurochemical manipulations of lateral amygdala: noradrenergic and dopaminergic mechanisms. *Brain Res* 249:95-101.
- Mackintosh NJ (1974) *The psychology of animal learning*. London: Academic Press.
- Malkova L, Gaffan D, Murray EA (1997) Excitotoxic lesions of the amygdala fail to produce impairment in visual learning for auditory secondary reinforcement but interfere with reinforcer devaluation effects in rhesus monkeys. *J Neurosci* 17:6011-6020.
- Mathe K, Toth A, Petyko Z, Szabo I, Czurko A (2007) Implementation of a miniature sized, battery powered electrophysiological signal-generator for testing multi-channel recording equipments. *J Neurosci Methods* 165:1-8.
- McLaughlin J, Powell DA, White JD (2002) Characterization of the neuronal changes in the medial prefrontal cortex during jaw movement and eyeblink Pavlovian conditioning in the rabbit. *Behav Brain Res* 132:117-133.
- Merchenthaler I, Stankovics J, Gallyas F (1989) A highly sensitive one-step method for silver intensification of the nickel-diaminobenzidine endproduct of peroxidase reaction. *J Histochem Cytochem* 37:1563-1565.

- Moruzzi G, Magoun HW (1949) Brain stem reticular formation and activation of the EEG. *Electroencephalogr Clin Neurophysiol* 1:455-473.
- Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* 47:419-427.
- Ongur D, Price JL (2000) The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cereb Cortex* 10:206-219.
- Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ (2000) Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. *Behav Neurosci* 114:42-63.
- Paxinos G, Watson C (1996) *The Rat Brain*, 3 Edition: Academic Press.
- Perez-Jaranay JM, Vives F (1991) Electrophysiological study of the response of medial prefrontal cortex neurons to stimulation of the basolateral nucleus of the amygdala in the rat. *Brain Res* 564:97-101.
- Petyko Z, Toth A, Szabo I, Galosi R, Lenard L (2009) Neuronal activity in rat medial prefrontal cortex during sucrose solution intake. *Neuroreport* 20:1235-1239.
- Pezze MA, Marshall HJ, Cassaday HJ (2015) Dopaminergic modulation of appetitive trace conditioning: the role of D1 receptors in medial prefrontal cortex. *Psychopharmacology (Berl)* 232:2669-2680.
- Pinault D (1994) Golgi-like labeling of a single neuron recorded extracellularly. *Neurosci Lett* 170:255-260.
- Schalomon PM, Robertson AM, Laferriere A (1994) Prefrontal cortex and the relative associability of taste and place cues in rats. *Behav Brain Res* 65:57-65.
- Siegel JJ, Kalmbach B, Chitwood RA, Mauk MD (2012) Persistent activity in a cortical-to-subcortical circuit: bridging the temporal gap in trace eyelid conditioning. *J Neurophysiol* 107:50-64.
- Skinner BF (1938) *The behavior of organisms: An experimental analysis*. New York: Appletton-Century-Crofts.
- Skinner BF (1953) *Science and human behavior*. New York: Macmillan.
- Thorndike EL (1911) *Animal intelligence*. New York: Macmillan.
- Totah NK, Kim YB, Homayoun H, Moghaddam B (2009) Anterior cingulate neurons represent errors and preparatory attention within the same behavioral sequence. *J Neurosci* 29:6418-6426.
- Toth A, Petyko Z, Mathe K, Szabo I, Czurko A (2007) Improved version of the printed circuit board (PCB) modular multi-channel microdrive for extracellular electrophysiological recordings. *J Neurosci Methods* 159:51-56.
- Vogt BA, Peters A (1981) Form and distribution of neurons in rat cingulate cortex: areas 32, 24, and 29. *J Comp Neurol* 195:603-625.
- Wagner AR, Brandon SE (1989) Evolution of a structured connectionist model of Pavlovian conditioning (AESOP). In: *Contemporary learning theories: Pavlovian conditioning and the status of traditional learning theory* (Klein SB, Mowrer RR, eds). Hillsdale, NJ: Erlbaum.
- Watanabe M (1996) Reward expectancy in primate prefrontal neurons. *Nature* 382:629-632.
- Yamamoto T, Ueji K (2011) Brain mechanisms of flavor learning. *Front Syst Neurosci* 5:76.
- Zilles K, Wree A (1995) Cortex: areal and laminar structure. In: *The rat nervous system*, 2 Edition (Paxinos G, ed), pp 649-685. London: Academic Press.

List of publications

Publications that serve as a basis for the dissertation

- Petyko Z, Galosi R, Toth A, Mate K, Szabo I, Karadi Z, Lenard L (2015) Responses of rat medial prefrontal cortical neurons to Pavlovian conditioned stimuli and to delivery of appetitive reward. *Behav Brain Res* 287:109-119. IF: 3,028
- Petyko Z, Toth A, Szabo I, Galosi R, Lenard L (2009) Neuronal activity in rat medial prefrontal cortex during sucrose solution intake. *Neuroreport* 20:1235-1239. IF: 1,805
- Mathe K, Toth A, Petyko Z, Szabo I, Czurko A (2007) Implementation of a miniature sized, battery powered electrophysiological signal-generator for testing multi-channel recording equipments. *J Neurosci Methods* 165:1-8. IF: 1,884

Other publications

- Galosi R, Hajnal A, Petyko Z, Hartmann G, Karadi Z, Lenard L (2015) The role of catecholamine innervation in the medial prefrontal cortex on the regulation of body weight and food intake. *Behav Brain Res* 286:318-327. IF: 3,028
- Kallai V, Toth A, Galosi R, Szabo I, Petyko Z, Karadi Z, Kallai J, Lenard L (2015) [MAM-E17 schizophrenia rat model]. *Psychiatr Hung* 30:4-17.
- Buzas P, Kobor P, Petyko Z, Telkes I, Martin PR, Lenard L (2013) Receptive field properties of color opponent neurons in the cat lateral geniculate nucleus. *J Neurosci* 33:1451-1461. IF: 6,747
- Toth A, Mathe K, Petyko Z, Szabo I, Czurko A (2008) Implementation of a galvanically isolated low-noise power supply board for multi-channel headstage preamplifiers. *J Neurosci Methods* 171:13-18. IF: 2,092
- Toth A, Petyko Z, Mathe K, Szabo I, Czurko A (2007) Improved version of the printed circuit board (PCB) modular multi-channel microdrive for extracellular electrophysiological recordings. *J Neurosci Methods* 159:51-56. IF: 1,884
- Hernadi I, Karadi Z, Vigh J, Petyko Z, Egyed R, Berta B, Lenard L (2000) Alterations of conditioned taste aversion after microiontophoretically applied neurotoxins in the medial prefrontal cortex of the rat. *Brain Res Bull* 53:751-758. IF: 1,758
- Petyko Z, Lenard L, Sumegi B, Hajnal A, Csete B, Faludi B, Jando G (1997) Learning disturbances in offspring of zidovudine (AZT) treated rats. *Neurobiology (Bp)* 5:83-85.
- Petyko Z, Zimmermann T, Smola U, Melzer RR (1996) Central projections of Tomosvary's organ in *Lithobius forficatus* (Chilopoda, Lithobiidae). *Cell Tissue Res* 283:331-334. IF: 1,740

Citable abstracts

- Petykó Z, Tóth A, Gálosi R, Szabó I, Máté K, Szabó I, Karádi Z, Lénárd L: Responses of rat medial prefrontal cortical neurons to Pavlovian conditioned stimuli and to delivery of appetitive reward. In: XV. Biannual Conference of the Hungarian Neuroscience Society Hungarian Academy of Sciences, Budapest, Hungary, 2015.01.22-2015.01.23.pp. 88-89.

- Tóth A, Petykó Z, Feldmann Á, Kállai V, Gálosi R, Karádi K, Máthé K, Szabó I, Karádi Z, Lénárd L: Single unit analysis during auditory sensory gating in medial prefrontal cortex in freely moving rats. In: XV. Biannual Conference of the Hungarian Neuroscience Society Hungarian Academy of Sciences. Budapest, Hungary, 2015.01.22-2015.01.23.p. 112.
- Buzás Péter, Kóbor Péter, Petykó Zoltán: A színlátás pályarendszerei SZEMÉSZET 151:(Suppl.) pp. 32-33.(2014)
- Kállai V, Gálosi R, Tóth A, Petykó Z, Ollmann T, Péczely L, Kovács A, Kállai J, Szabó I, Lénárd L: The MAM-E17 rat model of schizophrenia: Behavioral examinations. In: IBRO Workshop: International Brain Research Organization Workshop. Debrecen, Hungary, 2014.01.16-2014.01.17.Paper P77.
- Kóbor P, Petykó Z, Buzás P: Temporal frequency tuning and response phase of blue-ON cells in the lateral geniculate nucleus of the cat. In: IBRO Workshop: International Brain Research Organization Workshop. Debrecen, Hungary, 2014.01.16-2014.01.17.Paper P42.
- Petykó Z, Toth A, Galosi R, Szabo I, Mate K, Szabo I, Karadi Z, Lenard L: Neuronal responses of the rat medial prefrontal cortex during appetitive classical conditioning ACTA PHYSIOLOGICA 211:(697) p. 160.(2014) Joint meeting of the Federation of European Physiological Societies (FEPS) and the Hungarian Physiological Society. Budapest, Hungary: 2014.08.27 -2014.08.30.
- Tóth A, Petykó Z, Feldmann Á, Kállai V, Gálosi R, Karádi K, Máthé K, Szabó I, Karádi Z, Lénárd L: Single unit analysis of auditory sensory gating in the medial prefrontal cortex (mPFC) in freely moving rats. In: IBRO Workshop: International Brain Research Organization Workshop. Debrecen, Hungary, 2014.01.16-2014.01.17.Paper P95.
- Kóbor P, Petykó Z, Papp L, Allston MA, Buzás P: Testing a novel 7-channel deep brain microelectrode for parallel single unit recording in the cat thalamus In: Csillag András (szerk.) XIV. Conference of the Hungarian Neuroscience Society. 282 p. Budapest, Hungary, 2013. Paper P3.8.
- Petykó, Z., A. Tóth, R. Gálosi, I. Szabó, K. Máté, I. Szaó, Z. Karádi, L. Lénárd: Neuronal responses of the rat medial prefrontal cortex during appetitive classical conditioning. Acta Physiologica, Special Issue: Abstracts of the Joint Meeting of the FEPS and the HPS, 27-30 August, 2014, Budapest, C: Scandinavian Physiological Society, Published by J. Wiley & Sons Ltd. V211: Issue Suppl. S697: 62-184: P. 10.51., 2014. (DOI:10.111/alpha.12362)
- Kállai V., R. Gálosi, A. Tóth, Z. Petykó, T. Ollmann, L. Péczely, A. Kovács, J. Kállai, I. Szabó, L. Lénárd: The MAM-E17 rat model of schizophrenia: Behavioral examinations. IBRO Workshop, Debrecen 16-17. January, Hungary, P77, 2014.
- Tóth, A., Z. Petykó, V. Kállai, R. Gálosi, K. Karádi, Á. Feldmann, K. Máthé, I. Szabó, Z. Karádi, L. Lénárd: Local field potential analysis of auditory sensory gating in medial prefrontal cortex in freely moving rats. 8th FENS Forum of Neuroscience, July, 14-18, Barcelona, p.: 587, 2012.
- Tóth, A., Z. Petykó, V. Kállai, R. Gálosi, K. Karádi, Á. Feldmann, K. Máthé, I. Szabó, Z. Karádi, L. Lénárd: Auditory sensory gating local field potential analysis in the medial prefrontal cortex in freely moving rats. Clinical Neuroscience, 65(S1): 69-70, 2012.
- Petykó, Z., A. Tóth, R. Gálosi, K. Karádi, K. Máthé, I. Szabó, Z. Karádi, L. Lénárd: Reward predicting neurons in the rat medial prefrontal cortex. 8th FENS Forum of Neuroscience, July, 14-18, Barcelona, p.: 437, 2012.

- Petykó, Z., A. Tóth, R. Gálosi, K. Karádi, K. Máthé, I. Szabó, Z. Karádi, L. Lénárd: Neurons in the rat medial prefrontal cortex (mPFC) respond to reward predicting acoustic stimuli. *Clinical Neuroscience*, 65(S1): 53-54, 2012.
- Kóbor, P., Z. Petykó, I. Telkes, L. Lénárd, Á. Lukács, Á. Szél, P. Buzás: Mixed cone inhibition in colour cells of the cat retina. *Clinical Neuroscience*, 65(S1): 35-36, 2012.
- Tóth, A., Z. Petykó, R. Gálosi, K. Karádi, K. Máthé, I. Szabó, Z. Karádi, L. Lénárd: Auditory sensory gating multi unit and local field potential analysis in medial prefrontal cortex in freely moving rats. *Acta Physiologica*, 202(Suppl 684): 119-120, P86, 2011.
- Petykó, Z., A. Tóth, R. Gálosi, K. Karádi, K. Máthé, I. Szabó, Z. Karádi, L. Lénárd: Reward related single-unit activity changes in the rat medial prefrontal cortex. *Acta Physiologica*, 202(Suppl 684): 96, P69, 2011.
- Petykó, Z., A. Tóth, R. Gálosi, Z. Karádi, K. Máthé, I. Szabó, L. Lénárd: Reward related single unit activity changes in the rat medial prefrontal cortex. 8th IBRO World Congress of Neurosci. Florence, Italy, July 14-18, 2011, 14. Cognition and emotion, D347, 2011.
- Kóbor, P., Z. Petykó, I. Telkes, L. Lénárd and P. Buzás: Receptive field properties of colour cells in the cat lateral geniculate nucleus. 13th Meeting of the Hungarian Neuroscience Society, January, Budapest, Hungary, 2011. P.5-2, *Frontiers in Neuroscience*, (doi: 10.3389/conf.fnins.2011.84.00158)
- Gálosi, R., A. Tóth, Z. Petykó, L. Lénárd: Effects of catecholaminergic lesion of prelimbic – prefrontal cortex on cued discrimination task in rats. 8th IBRO World Congress of Neurosci., Florence, Italy D297, 2011. July 14-18, 2011, 13. Learning and memory, D297, 2011.
- Gálosi, R., A. Tóth, Z. Petykó and L. Lénárd: Operant cued discrimination task: effects of noradrenaline lesion of prelimbic cortex. *Acta Physiologica*, 202(Suppl 684): 35, P23, 2011.
- Gálosi, R., P. Pusztai, N. Hajnal, A. Tóth, Z. Petykó and L. Lénárd: Effects of catecholaminergic lesions of the prelimbic prefrontal cortex on sustained attention demanding task performance in rats. 13th Meeting of the Hungarian Neuroscience Society, January, Budapest, Hungary, 2011. P.1-29, *Frontiers in Neuroscience*, (doi: 10.3389/conf.fnins.2011.84.00164)
- Tóth, A., Z. Petykó, K. Máthé, R. Gálosi, L. Lénárd, I. Szabó: Auditory sensory gating investigations in medial prefrontal cortex. *Acta Physiol. Hung.*, 97(4): 482, 2010.
- Tóth, A., Z. Petykó, K. Máthé, M. Wlasitsch, R. Gálosi, L. Lénárd, I. Szabó: Methodological requirements of electrophysiological investigations of acoustic startle response. P7-25. *Frontiers in Neuroscience*, Conference Abstract: IBRO International Workshop 2010. doi: 10.3389/conf.fnins.2010.10.00265
- Gálosi, R., A. Tóth, Z. Petykó, L. Lénárd: Effects of local noradrenaline depletion in the medial prefrontal cortex and body weight regulation. P5-11., *Frontiers in Neuroscience*, Conference Abstract: IBRO International Workshop 2010. doi: 10.3389/conf.fnins.2010.10.00088
- Buzás, P., Z. Petykó, P. Kóbor, I. Telkes, L. Lénárd: "Blue-yellow" chromatic opponent responses in the lateral geniculate nucleus of the cat. *Acta Physiol. Hung.*, 97(4): 430-431, 2010.
- Gálosi, R., A. Tóth, Z. Petykó, L. Lénárd: Local noradrenaline depletion elicits enhanced dopamine activity in the medial prefrontal cortex. 12th Meeting of HNS, Budapest, January, 2009. P221. doi:10.3389/conf.neuro.01.2009.04.193
- Petykó, Z., A. Tóth, I. Szabó, R. Gálosi, L. Lénárd: Multichannel recording of medial prefrontal cortex neurons during glucose solution intake in freely moving rats. *Clinical Neuroscience*, 61(Suppl1): 51, 2008.

- Máthé, K., Z. Petykó, A. Tóth, R. Gálosi, I. Szabó, L. Lénárd: 64-channel recording of medial prefrontal cortex neurons during glucose solution intake in freely moving rats. *FENS Abstract*, Vol: 4, 225.7, p.: 633, 2008.
- Tóth A., Z. Petykó, I. Szabó, K. Máthé, M. Katona, A. Czurkó, R. Gálosi, L. Lénárd: Simple devices for multiple unit activity recordings in freely moving in rats. 39th Annual European Brain and Behaviour Society Abstracts, p.: 85. 2007.
- Tóth A., Z. Petykó, I. Szabó, K. Máthé, M. Katona, A. Czurkó, R. Gálosi, L. Lénárd: Simple devices for multiple unit activity recording in rats. *Acta Physiologica Hungarica* 94(4): 397-398, 2007.
- Petykó, Z., A. Tóth, I. Szabó, R. Gálosi, L. Lénárd: Firing rate patterns in medial prefrontal cortex neurons during glucose solution intake in freely moving rats. 39th Annual European Brain and Behaviour Society Abstracts, p.: 85. 2007.
- Petykó, Z., A. Tóth, I. Szabó, R. Gálosi, L. Lénárd: Recording of multiple unit activity in medial prefrontal cortex during glucose solution intake in freely moving rats. *Acta Physiologica Hungarica* 94(4): 385, 2007.
- Gálosi, R., G. Hartmann, Z. Petykó, A. Tóth, L. Lénárd: Effects of lesions of catecholamine terminals in the medial prefrontal cortex on dopamine and noradrenaline levels and on the regulation of body weight. 39th Annual European Brain and Behaviour Society Abstracts, p.: 114, 2007.
- Petykó, Z., R. Gálosi, L. Lénárd: Drug administration via reverse dialysis during juxtacellular recording of single unit activity in the rat amygdale. *Clinical Neuroscience*, 58. Suppl. 1: 76, 2005.
- Petykó, Z., P. Marton, Cs. Niedetzky L. Lénárd: Electrophysiological characterization and labeling with Neurobiotine of single amygdalar neurons. *Clinical Neuroscience*, 56(2): 70, 2003
- Petykó, Z., Cs. Niedetzky, L. Lénárd: Morphological characterisation of single medial prefrontal neurons responding to subcortical stimulation. *Acta Physiologica Hungarica*, 89(1-3): 212, 2002.
- Petykó, Z., Cs. Niedetzky and L. Lénárd: Morphological and electrophysiological characteristics of medial prefrontal neurons in the rat. *Neurobiology*, 9(3-4): 353, 2001.
- Petykó, Z., Cs. Niedetzky, I. Hernádi, L. Lénárd: Labeling of electrophysiologically characterised neurons with Neurobiotine in the rat prefrontal cortex. *Neurobiology*, 9(3-4): 246, 2001.
- Lénárd, L., Z. Karádi, I. Hernádi, Z. Petykó, B. Berta, R. Egyed and K. Várady: Feeding-associated gustatory information processing and taste aversion learning in the limbic system: Electrophysiological and behavioral investigations. *Chemosensory Information Processing. A Hungarian-Israeli Interacademy Workshop*, 4-5-September, 2000., Budapest. Abstract Book, P.: 17, 2000.
- Petykó, Z., I. Hernádi, Cs. Niedetzky and L. Lénárd: Extracellular recording and simultaneous biocytin labeling of neurons in the insular cortex of the rat. *Neurobiology*, 7(3): 374-375, 1999.
- Gálosi, R., A. Hajnal, Z. Petykó and L. Lénárd: Enhanced glucose preference after the lesion of dopaminergic terminals in the medial prefrontal cortex. *Appetite*, 31(2): 244, 1998.
- Lénárd, L., E. Kertes, G. Nagyházi and Z. Petykó: Enhancement of positive and negative reinforcement by Substance P. *Ann. Congr. of IBNS, Richmond, USA, Abstracts of IBNS*, Vol. 7: 31, 1998.
- Petykó, Z., L. Lénárd and R. Gálosi: Catecholaminergic innervation of the prefrontal cortex in the rat. *IVth Ann. Meeting of MITT, Debrecen, Hungary*, 1998.