The role of the medial prefrontal cortex in the auditory sensory-motor-gating mechanism

Ph.D. thesis

Attila Tóth

UNIVERSITY OF PÉCS
FACULTY OF MEDICINE

Theoretical Medical Sciences Ph.D. Program
Head of the Ph.D. School: Prof. Júlia Szekeres, M.D., Ph.D., D.Sc.
Head of the Ph.D. Program: Prof. Zoltán Karádi, M.D., Ph.D.
Tutor:
Prof. Imre Szabó M.D. Ph.D.
Prof. László Lénárd, M.D., Ph.D., D.Sc.

Pécs, 2019

List of abbreviations

ASR – Acoustic Startle Reaction

EMG – Electromiogram

EP – Evoked Potential

ERP – Evoked Response Potential

PSC – Primery Startle Circuit

fMRI – functional Magnetic Resonance Imaging

LFP – Local Field Potential

mPFC – medial Prefrontal Cortex

NAC – Nucleus Accumbens

OCD – Obsessive Compulsive Disorder

PCB – Printed Board Circuit

PET – Pozitron Emission Tomography

PFC – Prefrontal Cortex

PPI – Prepulse Inhibition

SPL – Sound Pressure Level

VCN – Nucleus Ventralis Cochlearis

VLL – Lemniscus Ventrolateralis

VTA – Ventral Tegmental Area

Introduction

The limited information processing capacity of the central nervous system makes it necessary to prevent the entry of irrelevant stimuli into the higher neurological centres. To achieve this, a number of mechanisms have been developed that effectively select the volume of incoming information separately and jointly. Sensory-motor-gate mechanisms are the core components of the brain's signal-processing mechanism, which are necessary to select the stimuli that are important to the individual. The filtered information will lead to the development of the organised behavioural process (Graham et al., 1975) (Blumenthal et al., 1996) (Swerdlow et al., 2000) (Fendt et al., 2001). With regard to the experimental examination of this process, perhaps with the greatest relevance, the startle reaction and its modified version, prepulsive inhibition (PPI) plays an essential role. In terms of modality, any strong sensory stimulus can generate a startle response. (In human studies, the form of the acoustic, the use of a tactile-stimulus (air-puff), is a predilection.) The most researched and best-performing form is the reaction induced by an acoustic stimulus. The acoustic startle reflex and its modified version of Prepulsive

inhibition show deviations in a number of neuropsychiatric conditions [schizophrenia, schizotype personality disorder, Huntigton's disease, Obsessive compulsive Disorder, OCD, Tourette syndrome, Attention deficit syndrome (FDD, Attention deficit disorder, ADD)] (Swerdlow and Geyer, 1998). The common denominator of the aforementioned diseases is that the decrease in the filtration of incoming sensory information will result in a change in the given motor, along with the cognitive response. A number of metodikes have been developed to test the sensory-motor capation deficit. However, on the basis of the literature review, it is believed that the most accurate results for the examination of the neurological lesions of the absence of inhibition can be served by prepulsive inhibition (PPI). The malfunction of the gating mechanism can contribute to sensory overcharge or flooding, which ultimately leads to cognitive fragmentation, thought, perception disorder and other psychotic symptoms (Braff and Geyer, 1990); (Geyer et al., 1990); (Braff et al., 2001a). The startle reaction is an extremely fast, reflex reaction, suggesting that the relationship between only a few nerve cells creates a distinctive response. However, in addition to the simple primary structure, it is also necessary to observe the approach of stacking mechanisms when designing the appropriate reaction. For example, attention, learning and other cognitive processes, of which the prefrontal cortex is crucial. Prepulsive inhibition is an extremely complex process in itself, which also plays a role in cortical areas, primarily the prefrontal cortex, which performs important regulatory functions in the process. This is indicated by experiments leading to a PPI change following the lesion of the area (Koch and Bubser, 1994); (Japha and Koch, 1999); (Schwabe et al., 2004, Schwabe and Koch, 2004). The extremely complex structure and resultant role of the prefrontal cortex, as well as the very rapid head movement during the startle reaction, which causes a significant movement artefact during registration, is a major obstacle to Electrophysiologic Methods to monitor the operation, the modification and the modified version of the nerve cells in this area, below PPI. In carrying out our tests, we sought to register and analyse neuronal changes in this area.

Objectives

Our primary goal in relation to current research was to make it descriptive and to investigate the parametric properties of sensory gating. For this purpose, the development of a specially designed multiunit recording system, which is suitable for the collection of single-unit activity with chronic electrodes planted to the medial prefrontal cortex (mPFC), freely moving animals. We believed that registering rapid head movements could provide additional usable data to understand the neurologic background of the reaction of startle and its modified version of Prepulsive inhibition. We assumed that at both neuronal activity levels we had a demonstrably stable state. The ultimate goal was to test the sensory gating process at the single-unit level. Our hypothesis was that, due to prepulsive inhibition, the decrease in the degree of gating stimulus caused a change in the neuronal response to mPFC. By registering the single unit activity, you can obtain an image of the special operation of mPFC in regulating the sensory gating mechanism.

Goals

• Aimed at developing a microdrive with advantages (such as ***compact structure, low weight and small size, which does not limit the normal behaviour of an adult rat and is easily adaptable to different experimental situations, ensuring extremely stable unit recording***, etc.) They are capable of carrying out appropriate single-unit recording in the course of the startle, on freely moving, behaving animals.

• I have further targeted the study of the ***startle reaction*** and its ***modified version, prepulsive inhibition (PPI) with multi-unit technique (multichannel, tetrode recording), from rat medial prefrontal cortex (mPFC)***.
• Although not included in my original objectives, the ***development of a new method to measure the degree of the startle reaction during our experiments***. For this purpose, in our research team we have developed the ***3D head accelerometer***, ***with which we are able to determine much more accurately the head movements than before***.

Methodological results (technical developments)

PCB microdrive

The drive, as the original PCB drive, is fabricated from PCB modular elements, brass screws and nuts, brass spacers, and flexible fused silica tubes. Two #00-90" Brass Round Head Screws (J.I. Morris Co., Southbridge, MA, USA) are surrounded by the PCB assembly (horizontal white layers). Long and short brass spacers (3M Board Mount Interconnect Products; DigiKey Corp., MN, USA or RS Export, RS Components Ltd., Corby, Northamptonshire, UK) are going through the PCB layers vertically. The length of the spacers and screws can be custom-cut depending on the experimental condition.
In this version of PCB microdrive, the electrodes are carried by small PCB plates that are soldered to the hexagon-shape nuts. When turning the screws, the nuts are going up or down together with the small PCB plates. Parallel to the screws, long and short brass spacers are holding the whole drive together through all five layers of PCB boards. The long brass spacer rods can extend optionally above the screw level with an additional 'covering top' PCB, that is smaller in diameter. In this arrangement, with the additional covering top PCB, it is possible to load short and long electrodes (in short and long thin silica capillaries, see later) into the microdrive. This way, the long electrodes are extendin well above the screw level, but they are protected by the covering top PCB. The upper end of the electrode wires are reaching the electrode interface board in two levels, in an upper level and a lower level, the top view of six circular PCB boards used for fabrication of the modified PCB microdrive. The conventional circular arrangement of the screws was used, that enables 16 screws in the microdrive. The circular arrangement of the screws was one version of the original PCB microdrive described before (Szabo et al., 2001), and this is the usual arrangement for the microelectrode driving screws in several existing multi-channel microdrive designs (Neuralynx Inc., Tucson, AZ, USA). The advantage of this arrangement is that the microelectrodes are easily collected to the electrode guiding grid as they are equidistant from the centre of the drive. In our typical arrangement we used the half of each of the circular PCB plates, with holes for 8 screws. This results in a 32-channel (half) microdrive with eight tetrodes and a convenient connection to the improved electrode interface board. As it was mentioned before, in this version of the PCB microdrive the electrodes are carried by small PCB plates. These small PCB plates slide along the space bars up-and-down inside the large PCB assembly. To improve the stability of the electrode advancing mechanism, long spacer bars are inserted through the middle of the small PCB plates, and short spacer bars hold them from both sides at the screw. In this arrangement, even the slightest rotations of the small PCB plates are prevented by the spacer bars when the electrode positions are adjusted by turning the screws. The plates smoothly slide up-and-down along the spacer bars, without any rotation (at direction changes). This produced an improved stability for the electrode advancing mechanism, that is clearly advantageous for chronic unit recordings, e.g. from freely moving animals. Two 'thin' flexible fused silica capillaries (TSP100200, 100 and 200 _m inner and outer diameters, respectively; Polymicro Technologies, Phoenix, AZ, USA or Optronis GmbH, Kehl, Germany, EU) are glued to one small PCB plate

that moves up-and-down with the hexnut. The thin silica capillary contains the micro-wire for electrophysiological recording (e.g. wire tetrodes) from four 25 _m H-ML insulated Ni–Cr wires (Stablohm 675) or 12 _m H-Formvar insulated (Butyral bond coated) tungsten wires (California Fine Wire, CA, USA). The tip of the two electrodes in the brain can be on the same level or in small vertical separation. The upper end of the electrode wires will reach the electrode interface board in two well separated levels, as mentioned before. There are also 'thick' flexible fused silica capillaries (TSP250350, 250 and 350 _minner and outer diameters, respectively) in this new microdrive design. These thick capillaries are glued to the lower two PCBs. They tunnel, guide and protect the thin silica capillaries containing the electrode wires. The lower end of the thick silica capillaries are glued side-by-side to each other and in this way they can serve the same purpose as the 'electrode guiding grid' in the previous PCB microdrive design (Szabo et al., 2001). The thick silica capillaries guide the proper movement of the thin silica capillaries with the electrodes when they bend on the way down to the target area. This keeps the microelectrodes constantly in a stable position and yields stable recordings when the animal is freely moving. Obviously the silica capillaries can be replaced by adequately sized metal tubing if needed. After the microdrive is assembled, the thin silica tubes are loaded with the recording electrodes. Usually in our case tetrodes made from 25_m H-ML insulated Ni–Cr wires (Stablohm 675, California Fine Wire, CA, USA) are pushed through the thin silica capillaries under microscopic control. When both short and long thin silica capillaries are used, the two levels help the separation of the wires while connecting them to the electrode interface board. In the case of the 8-screw microdrive, when the circular PCBs are separated into two half discs, the electrode interface board conveniently fits to the flat side of the microdrive. In this arrangement, the extended part of the long bar spacers with their covering top PCB and the electrode interface board protect the long electrode shafts from damage. Electrical shielding against external artifacts can also be achieved by connecting the long bar spacers to GND. When the microdrive is loaded with short and long recording electrode wires, they can reach the electrode interface board at an upper and a lower (screw) level. Partly because of this reason, the electrode interface board of the previous PCB microdrive was redesigned. The new design uses a different type of linear double row micro-connector (0.050__ Grid Double Row Interconnects from Mill-Max Manufacturing Corp., Oyster Bay, NY, USA or Preci-Dip brand connector, series 853, 100 pins, double row, Felco Electronics Ltd., UK, EU), and the bottom part of the PCBs have prefabricated holes for these high density microconnectors.

The first design is for 32 channels, and this is a two layer PCB (25mm×24 mm). This can be fit, e.g. to an 8-screw drive with eight tetrodes. The advantage of this design is that there are three pre-plated holes for every electrode wire. These pre-plated holes are equally connected to the same connector pin. This way, every electrode wire can be connected to any of its three pre-plated holes, so the electrode wires can be arranged on the electrode interface board in a convenient manner. Having two levels of electrode wires from electrodes to electrode interface board makes it possible to attach two tetrodes to each up-and-down moving electrode plate. In this case, the 8-screw microdrive can contain 16 stereotrodes that fit to a single 32-channel electrode interface board; or with tetrodes it becomes a 64-channel microdrive and one can use two 32- channel electrode interface boards or a single 64-channel one. The second design is a 64-channel electrode interface board, using a four layer PCB (25mm×23 mm). The bottom part of the PCB has prefabricated holes for the two double row 32 pin high density Preci-Dip micro-connectors. In this interface board there is only one pre-plated hole for every electrode wire on the top part of the board. This interface board is convenient for the 8-screw microdrive, with two tetrodes per moving plate, as it was mentioned before, or with the full cylinder 16-screw microdrive with 16 tetrodes (64 channels). After the proper electrode interface board is attached to the microdrive, the upper ends of the electrode wires are cleaned and soldered to the electrode interface board. The soldering can be done by microsolder under the stereomicroscope, or

alternatively the micro-wires can be glued to the interface board by a tiny drop of nickel print or silver print (GC electronics, Rockford, IL, USA). A major advantage of the new electrode interface board design is that the interface board is sitting on the side of the microdrive, and the micro-connector is at the bottom of the interface board. This way the multi-channel preamplifier board does not extend higher than the top of the microdrive, and would not collect mechanical and electrical artifacts. In fact, a properly designed 32-channel preamplifier or two of them (Noted Bt., P´ecs, Hungary, http://www.users.atw.hu/ braintelemeter/headstage-32.htm) can conveniently sit next to the microdrive and they are protected by the electrode interface board and the volume of the microdrive itself. This arrangement that the electrode interface board is on the side of the microdrive opens up a further combination of microdrive arrangement. It is possible to stack two microdrives on top of each other, and we called this a double stage PCB microdrive. We slightly had to modify the PCB design to make room for the pass through silica capillaries at the bottom part of the double stage PCB microdrive. This basically means some additional nonplated holes both on the main PCB discs and also on the small moving PCB plates. The double stage PCB microdrive is not significantly higher than an average commercially available multi-channel microdrive and it is an elegant demonstration of the modularity of the PCB microdrive design. During the surgical implantation of the PCB microdrive a spacer bar is soldered parallel to the others and this extends above the whole drive. This spacer bar is held by the stereotaxic positioner for surgical implantation and is cut afterwards.

After several machine screws are tapped into the skull to serve as anchors for the acrylic, a hole is drilled above the target area into the skull and the dura is cut under the dissection microscope. The 32-channel PCB microdrive just before implantation (height: 4.5 cm; weight: 7.5 g). The upper part of the thick silica capillaries are embedded in acrylic, the long bar spacers are bent to conveniently fit to the top of the skull. The electrode assembly is lowered into the brain above the target area and the exposed brain surface, the electrodes and the extending thin and thick silica capillaries are covered with warm (56 ◦C) mixture of paraffin: paraffin oil (1:1). At room temperature this mixture hardens, so it protects the electrode assembly but also helps in the smooth movement of the capillaries. Later, several layers of acrylic are applied to the skull, so the bottom parts of the long bar spacers are embedded in acrylic. The microdrive and head connector are protected by micropore tape. ThePCBmicrodrives can be reused as only the end of the long bar spacers and the silica capillaries are embedded in acrylic. The acrylic can be dissolved (in chloroform) or the embedded end of the long bar spacers can be cut off and re-extended by soldering a small piece of spacer to it. After replacing the thick silica tubing the microdrive can be reloaded by new thin silica capillaries and electrodes. For free download of GERBER and NC drill files or further information about the PCB kits contact szaboimre@yahoo.com.

Discussion

A major modification of the improved version of PCB microdrive is that the electrodes are moved by small moving PCB plates that are soldered to hexagon-shape nuts and not merely by the hexagon nuts. In the original PCB microdrive design the nuts may have occasionally rotated when the screw turned. In the improved version of the recent design there is a small PCB plate that can move up-and-down. The hexagon-shape nuts are carried by these small plates since the nuts are soldered to them. The spacer rods are inserted through small drilled holes of these small PCB plates. In this way, the rotation of both the small PCB plates and the nuts are completely eliminated and the plates with the nuts are forced to move either up or down. This results in improved stability for the electrode advancing mechanism that is clearly advantageous for chronic unit recordings from freely moving animals. Further advancement of the new PCB

microdrive design that not only one but two flexible fused silica capillaries can be glued to one small moving PCB plate. This is a useful solution in many experimental situations where the target area is in one level, but the local field activity (local EEG) can be recorded from above or under the target area. This arrangement (two tetrodes to one advancing screw) is used, e.g. in some place cell recording experiments with the original O'Keefe drive design (Huxter et al., 2003). When there is a significant vertical separation between the tips of the electrodes, then there is good chance for the upper electrode to record cells from the layered target area after the lower electrode has passed through the same layer. An additional improvement of the PCB microdrive design was substantiated by the extensive use of fused silica capillaries. Not only one diameter of fused silica capillaries is used but two, in the way that the thin silica capillary can go into the thick one, and smoothly slide in it. This way the thick silica capillary protects the moving thin silica capillary that contains the tetrode electrode. This eliminates the need of the electrode guiding grid, and the thick silica capillaries can be freely collected above the target area or grouped above several target areas by simple cotton threads. The application of thin silica capillaries has an additional advantage. Multi-electrode design can produce signal attenuation if the saline pool around the insulated electrodes covers a large area and the wall of the electrode insulation is less than 10–30 _m (Gross, 1979). When the end of the silica capillaries is blocked by a drop of glue and no saline can cover the electrode wires inside the silica capillaries then the attenuating effect of electrode shunt capacitance becomes negligible and the proper signal transfer is ensured. The application of the PCB covering top protects the long thin silica capillaries extending from the top of the microdrive, and this makes it possible to connect the upper end of the electrode wires at two levels, to the improved electrode interface board. The improved electrode interface board is located on the side of the microdrive, and contains several pre-plated holes for the electrode wires. These holes are connected to the bottom part of the interface board where there are prefabricated holes for the reception of one or two double row 32 pin high density microconnectors. A major advantage of the new electrode interface board arrangement at the side of the microdrive is that the multichannel preamplifier board does not extend above the microdrive. This way, the preamplifier board does not collect mechanical or electrical artifacts, as it is protected by the electrode interface board and by the volume of the microdrive itself. A further advantage of the new electrode interface board arrangement is that it is possible to put two microdrives on the top of each other. With a slight modification of the PCB design we created a double stage PCB microdrive that is an elegant demonstration of the modularity of the PCB microdrive design. ***In conclusion, with the application of the PCB microdrive design, one can easily create prefabricated PCB kits from which anybody can build a precisely assembled multi-channel microdrive. The PCB microdrive will be very precise because of the high standards of the PCB manufacturers. On the other hand, assembling the microdrive from PCB kits is easy and it does not require specific skills or sophisticated machining tools.***

3D Head Accelerometer Tool

Precise timing measurement of motor responses is extremely relevant in many neuroscience researches, especially where electrophysiological testing is carried out. Using miniature accelerometer sensors, you can accurately detect animal behavior and the response rate. As a result, it provides an extremely useful tool for measuring the behaviour of freely moving laboratory animals. The ultra-fast reflex response is registered with a 1 ms resolution, amplified and digitilized signal. The motor component of startle requires a high frequency value (1000 Hz sampling frequency) and a range of high dynamic analog to digital conversion (12-bit). The commonly used startle measuring devices are based on a force meter or accelerometer sensor. The experimental animal is placed on a Plexiglas sheet in the soundproofed box. The normal behaviour of the animal is significantly restricted, as the free movement can be captured in a significantly different magnitude of startle responses. In the registration of the startle, the data of the 100-200 msec time window following stimulation is considered to be authoritative for the reaction. Taking these problems into account, our research team has developed a tool for measuring startle reaction based on 3d accelerometer adaptable to PCB microdrive. Originally a 32-channel pre-amplifier, the accelerometer features a 24-bit analog-to-digital conversion in addition to the previously mentioned 1000 Hz rezolution. From the 32 signal transmission channel, 4 channels are "sacrificed" for the 3-channel accelerometer and microphone. With the help of the microphone, the initial moment of the sound stimulus became highly accurate, and we used it as a trigger to evaluate our results later. With a miniature accelerometer sensor, the 28-channel Preamplifier gives us an opportunity to measure the head accelerations of the three directions of the space in a highly precise way. In addition to the exact 3-way measurement, the time ("timestamp") of the start of the stimulation can be determined with high precision using the microphone band. The tool we have developed was tested in various experimental situations, such as Pavlovi conditioning (Petyko et al., 2015), during the startle reaction (Toth et al., 2017). Pasquet and his colleagues came up with a similar outcome as the accelerators (Pasquet et al., 2016) that I would like to introduce later. The 3 components, the acceleration factor from the 3-direction of the space, and the evaluation program developed by them, could create a more accurate picture of the head movements used in their experiment to give the Pavlovi-fear conditioning conduct investigation. The authors underline the importance of spatial data, which makes it even easier to understand the interpretation of many other behavioural paradigm.

On this basis, we can say that the preamplifier with the 3D accelerometer that we have developed, which is created to measure the head accelerations in the process, is an objective method of measuring the chronic, freely-moving rats of startle reaction is much more precise than the previously used methods of the test.

Electrophysiologic Results

Materials and methods

Subjects: Three adult, 4 month old male Wistar rats (weighing 400–450 g) Varty and Higgins, 1994) were used in the present experiments. Individually caged animals were kept on 12-12-h light/dark cycle (light on at 6 a.m.) in a temperature and humidity controlled (24 ± 2◦C) vivarium. Standard laboratory food pellets (CRLT/N, Charles River Labo-ratories, Budapest, Hungary) and tap water were available adlibitum. Body weights were measured daily.

Electrode implantation

Prior to learning behavioral tasks, printed circuit board-based (PCB) microdrives loaded with eight tetrodes were implanted by stereotaxic guidance above the medial part of the PFC (details see later) (Toth et al., 2007; Petyko et al., 2009). To do so, rats were anesthetized with sodium-pentobarbital solution (60 mg/kg Nembutal, Phylaxia-Sanofi, Hungary) followed by i.p. atropine injection (2 mg/kg EGIS,Hungary). Skin was removed from the upper surface of the skulland anchor screws were inserted and driven into the bone. One of them was used as ground and another one as reference electrode. Burr holes were made and the PCB-microdrives were positioned by means of a micromanipulator above the mPFC (AP: 2.7 mm from bregma and ML: 0,8 mm according to the rat brain atlas of Paxinos and Watson (1996)) In this position thick silica tubes servingas guiding tubes for the tetrodes, reached the brain surface above the mPFC. All tetrodes were lowered approximately to the upperborder of the prelimbic area.

Behavioral procedure and equipment

Recording of behavioral actions and multiple unit activity has been started after a recovery period of 2 weeks. Experiments were performed in a sound attenuated 40 × 40 × 40 cm operant box during the light period. Auditory stimuli were presented via a dual piezo speaker (MPT-177) mounted on the roof of the chamber. The distance between the sound source and the rat head was 60 cm. We used two different trial types during the experiments. The first one was the startle/pulse alone trial condition (application of acoustic stimulus with 120 dB noise burst, presented for40 ms), the second one was the prepulse + pulse trial condition (prepulse stimulus, 75 dB noise burst, presented for 20 ms, 160 msbefore the startle stimulus). The sound pressure level was measured with validated sound pressure meter (ExTech EN300). Otherwise, the session began with a 5-min acclimatization (stan-dard PPI paradigm) period during which broadband white noise was presented at 65 dB through a speaker. Next 48 stimulation were presented, each containing both trial types in pseudorandom order (24-24 startle/pulse alone and prepulse + pulse trials in pseudorandom order). For the analysis we used the first 24 trials of each stimulation types. Intertrials intervals were at the pseudorandomly distributed range of 30–150 s. The whole experiment lasted for 9 days, in non-consecutive manner daily one session was performed. Behavioral testing, presentation of the different sound stimuli were controlled by the LabCommander software (NotedBt., Pécs, Hungary). Sound stimulation was generated by playingof audio (.wav) files using the sound card of the computer.

Data recording and spike sorting

For electrophysiological recording of neuronal activity and head acceleration, a miniature 32-channel head stage amplifier (NotedBt., Pécs, Hungary) with a homemade 3D-accelerometer (differential capacitive accelerometer: low power consumption, large output level, and fast

response to motions) invented by A. T.was plugged into the socket at the interface board on the micro-drive. Tetrodes were moved on after each session, obligatory noted for the subsequent reconstruction of recording sites. Wide-band signals (0.1 Hz–18.75 kHz) from 8 tetrodes (Startle 28 + 4channel preamplifier, Noted Bt., Pécs, Hungary), and events were recorded continuously by means of a 24-bit, 64-channel low volt-age AD converter (LVC-64, Noted Bt., Pécs, Hungary). Spikes were extracted after automatic threshold detection (power higher than 5 times the standard deviation from the baseline), and reconstructed to 37.5 kHz by using the principles of the sampling theorem (Csicsvari et al.,1998). Employing a principal component analysis, a 12-dimensional feature vector was created for each spike (first three principal components for each channel from a tetrode) (Abeles and Gerstein, 1988; Csicsvari et al., 1998). Spikes from putative individual neurons were segregated using automatic clustering software. Automatic clustering was performed by means of the KlustaKwik software (Harris et al,. 2000). Only units with clear symmetric gapin their autocorrelation, units with a clear refractory period (<3 ms, determined on the basis autocorrelograms) and well-defined ovalshaped cluster boundaries were used for further analysis. Cross-correlation histograms of all possible pairs recorded from a given tetrode probe were calculated by means of the Klusters software (Hazan et al., 2006). In case of symmetrical gap in the center of bins indicated that the initial clusters represented activities of the same unit clusters, so these clusters were merged.

Data analysis

The head acceleration during startle/pulse alone and pre-pulse + pulse trials was measured continuously (in a 250 ms time interval before and after the onset of startle reaction). Averaging of maximal head accelerations were extracted off-line and analyzed using t-test. Significance level was set to $p < 0.05$. Peristimulus time histograms (PSTHs) from 24 consecutive stimuli were calculated off-line by a PC. The statistical analysis (off-line analysis of data, generation of raster plots and PSTHs) was performed using MatLab and shell scripts written in Linux. PSTHs were used to detect single neuronal responses timelocked to events related to onset of startle/pulse alone or prepulse + pulse.The stimulus onset was used to detect neuronal responses relatedto the stimuli elicited behavioral actions. In each PSTH, time 0 wasset at the moment of the startle stimulus onset. Because of possible modulation of the firing rate related to the appearance of prepulse stimuli or ongoing behaviors, the time range -4 to -2 s relative to acoustic cue onsets was used to calculate baseline firing rate. In order to detect significant activity changes during thetwo different trial conditions (i.e. prepulse + pulse and startle/pulsealone), the time range of -2–2 s was used as test period. PSTH-s were calculated with a bin size of 20–200 msec. For each bin, the z-score was calculated from an approximate Poisson distribution ofthe expected firing rate. If the z-score was $\geq +2.36$ or ≤ -2.36 in atleast 3 consecutive bins the neuron was considered as responding to the event ($p < 0.01$) (Totah et al., 2009). For the analysis of population activ-ity, the z-scores of each responding unit were averaged. One-wayANOVA was performed with time and trial condition as main fac-tors. The significance level was set to $p < 0.01$. Whenever indicated by ANOVA, Tukey's HSD test was used for post hoc analysis where the level of significance was set at $p < 0.05$.

Histology

After the last experiment, rats were deeply anesthetized with Urethane and then perfused with 0.9% saline solution followed by a 10% solution of phosphate buffered formalin. After perfusion, thebrains were removed and stored in the perfusion solution for morethan one week. The brains were then sliced into 50 μm coronal sections in a microtome. The relevant sections

were mounted on glass slides. Toluidine blue histological staining was performed to detect electrode tracks. Electrode tracks and recording sites were reconstructed later with light microscopic evaluation.

Results

Histology

Electrode tracks of the eight tetrodes were located within the anterior-posterior position range of 2.2–3.2 mm from Bregma in all three rats (Paxinos and Watson, 1996). The reconstructed recording sites are demonstrated in Fig. 1. All recording sites were localized within the mPFC 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) according to the rat stereotaxic atlas of Paxinos and Watson (1996).

Head acceleration measurement during startle and PPIbehaviors

Quantitative determination of magnitude of the startle responses was based on the data of the head acceleration measurements. The maximum head acceleration values observed inthe first 100 ms after the startle stimulus were analyzed in bothparadigms. The prepulse stimulus preceded the onset of the startle stimulus by 160 ms. The predictive prepulse stimulus itself did not elicit startle response. When the peak of head acceleration during the two different trial conditions were compared with startle responses, significant difference was detected ($p < 0.05$). The grand average maps showed that in 13–18 ms time windows the amplitudes were significantly greater in case of the startle alone than in the PPI stimulation condition.

Neuronal responses

One hundred and forty-six (50.8%) of the altogether 287 detected neurons in the mPFC changed significantly their firing rate to at least one of the two types of stimulation conditions ($|z| > 2.36$; $p < 0.01$). Most of the neurons responded to both types of stimulations (startle/pulse alone and prepulse + pulse), whereas all neurons changing firing rate to the prepulse + pulse stimulation responded to pulse alone as well. The recorded single units were grouped in 3 types according to their response characteristics to the prepulse and pulse stimulation (short, medium and long duration units, respectively). One type of the units, the short duration group included neurons displaying short duration excitatory firing rate changes lasting for less than 50 ms. The prepulse + pulse related excitatory short responses were further subdivided into two subpopulation:(a) the ‘equal’ subpopulation, neuron activities with approximately identical same responses to both types of tones (prepulse, pulse); and (b) the ‘unequal’ subpopulation, neuron activities with different patterns to the two types of tones (this latter subpopulation was further divided into 2 subgroups [unequal prepulse, unequal startle]). A second major type of neurons, called medium duration units were found to change their firing rates with a short latency to the onset of the startle tone and lasting for more than 50 ms, but for less than 1 s. The third category of neurons, the long duration units were those that changed their firing rates with a long latencyto the onset of the startle tone and that lasted for more than 1s.

Short duration responses

Pulse alone

Forty-nine (17.1%) of the 287 recorded neurons changed their activity to pulse tones during the pulse alone trialtype.

Prepulse + pulse

Twenty-nine (10.1%) of the 287 recorded neurons changed their firing rate to pulse tones during pre-pulse + pulse stimulation. Among these 29 neurons, 10 were classified as short duration of the equal subpopulation, while 19 cells as short duration of the unequal subpopulation. In this latter subgroup, 2 neurons were found, which showed higher activation to prepulse than to pulse stimuli [unequal prepulse].

Population activity

The population activities of the 17 neurons exhibiting unequal excitatory responses during pulse alone [unequal startle], pulse stimuli of prepulse + pulse situation and prepulse stimuli of prepulse + pulse situation demonstrated complex response characteristics. There was a significant main effect due to factor time ($F(19.1020) = 10.57$, $p < 0.01$) and stimulus type ($F(2.1020) = 16.53$, $p < 0.01$). Furthermore, there was a significant interaction between stimulus type and time ($F(38.1020) = 1.98$, $p < 0.01$).The population activities of the 10 neurons exhibiting equal excitatory responses during the pulse alone, pulse stimuli of the prepulse + pulse situation and prepulse stimuli of the prepulse + pulse situation similar responses in all three conditions. There was a significant main effect due to factortime ($F(19,480) = 23.71$, $p < 0.01$) and stimulus type ($F(2480) = 6.9$,$p < 0.01$). There was, however no significant interaction between stimulus type and time. Due to the low number (2) of neurons [unequal prepulse], population activity was not calculated in case at the two unequal,prepulse dominated units.

Medium duration responses

Pulse alone

Eighteen neurons (6.27%) of the 287 changed their firing rate to pulse tones during pulse alone trial types.

Prepulse + pulse

Thirteen (4.53%) of the 287 neurons changed their firing rate to pulse tones during the prepulse + pulsetrial types.

Population activity

The population activity of the 13 neurons exhibiting excitatory responses during the pulse alone, pulsestimuli of prepulse + pulse situation and prepulse stimuli of pre-pulse + pulse situation supported the notion to distinguish this group from other types of neurons in the mPFC. There was a significant main effect due to factor time ($F(19,720) = 19.45$, $p < 0.01$) and stimulus type ($F(2720) = 2.04$, $p < 0.01$). There was, however, no significant interaction between stimulus type and time.

Long duration responses

Pulse alone

Seventy-nine (27.5%) of the 287 recorded neurons changed their firing rate to startle tones during the pulse alone trial type. Sixty-two (21.6% of the 287) of these neurons significantly increased, while 17 of 287 (5.92%) neurons decreased their firing rate during the trial.

Prepulse + pulse

Twenty-five (8.71%) of the 287 recorded neurons changed their firing activity to pulse tones during the pre-pulse + pulse trial types. Seven (2.44% of the 287) of these neurons significantly increased their firing rate above, whereas 18 (6.27%) neurons decreased their firing rates below the baseline level during the prepulse + pulse situation at least for more than 1 s.

Population activity

The population activity of the 7 neurons exhibiting excitatory responses during the pulse alone, pulse stimuli of prepulse + pulse situation and prepulse stimuli of pre-pulse + pulse situation further supported the distinctionof these cell types. There was a significant main effect due to factor time ($F(34,1155) = 15.78$, $p < 0.01$) and stimulus type ($F(2,1155) = 1.02$, $p < 0.01$). There was no significant interaction between stimulus type and time. The population activity of the 18 neurons exhibiting inhibitory responses during the pulse alone, pulse stimuli of prepulse + pulse situation and prepulse stimuli of prepulse + pulse situation gave additional insights to distinct characteristics of this group of the cells. There was a significant main effect due to factor time ($F(34,1260) = 10.35$, $p < 0.01$) and stimulus type ($F(2,1260) = 0.22$, $p < 0.01$). There was no significant interaction between stimulus type and time.

Discussion

The present experiments had two main goals: one of them was to obtain unprecedented information on the startle response and the PPI by recording single neuron activity in the mPFC in relation to pulse alone or prepulse + pulse stimulation conditions in freely moving rats, while the other was to invent a new method to objectively measure startle reaction. With respect to the former, according to our best knowledge, no other studies have examined the effects of startle and PPI by single unit recording technique; with respect to the latter, our findings indicate that the preamplifier with the 3D accelerometer provides a highly accurate way to objectively measure startle reaction.The prefrontal cortex, at the top of the sensorimotor cortical hierarchy, has already been considered to be essential in sensori-motor gating processes. This limbic forebrain cortical region has a key role in planning and executing actions at the highest level of cognitive capabilities. The PFC is intimately involved in working memory (i.e. the temporary storage of information) and its activity appears to serve a kind of "buffering" for various internal modulatory actions. Reportedly, it also plays role in attentional processes and memory consolidation as well. These various functions and the central regulatory roles in motivational and learning processes (Cardinal et al., 2002) or e.g. food and fluid intake behaviors (Hernadi et al., 2000; Nagy et al., 2012) are reflected by the extensive reciprocal connections of the mPFC with several other brain regions. The mPFC receives inputs from the medio-dorsal tha-lamus (MD) (Uylings and van Eden, 1990), the ventral tegmental area (Thierry et al., 1973), various parts of the basal ganglia (Groenewegen et al., 1997), the lateral hypothalamic area (Kita, 1978), the amygdala (Krettek and Price, 1977; Swanson, 1981; McDonald; 1987, 1991) and the hippocampus (Swanson, 1981; Jay et al., 1989). It is also innervated by other cortical areas such as by the paralimbic cortex (enthorinal and perirhinal cortices) and also the somatosensory and motor cortices. The mPFC sends projections to the mediodorsal thalamus, the hippocampus, the basolateral amygdala (BLA) and to wide areas of the basal ganglia, thus, it contributes to the formation of the cortico-striato-pallido-thalamo-cortical loops (Krettek and Price, 1977; Groenewegen, 1988; Alexander et al., 1990; Groenewegen et al., 1990; Vertes, 2004). It also pojects directly to the ventro-lateral periaquaeductal gray (vIPAG), a neuronal structure involved in the genesis of freezing responses (Vianna et al., 2001). In addition to the a forementioned variety of its functions, the mPFC is also known to be involved in coordinating the neural activation of other cortical and subcortical areas. By means of this "orchestrating" attribute, the mPFC plays an outstanding role in the selection of the relevant information from a chaos of unimportant background "noise". This is necessarily achieved by various gating mechanisms whose examination requires well designed experimental protocols. The PPI is an important practical method for investigating sensori-motor gating processes. It appears to be a useful tool for studying and understanding the neural basis of sensory gating deficits which are important causal factors leading to characteristic symptoms of such neuropsychiatric disorders as, e.g. schizophrenia is. It was also shown to have strong predictive validity to create new

hypotheses through which new drug targets can be approached (Braff et al., 2008). PPI is thought to be regulated by a prefrontocortico-limbic-striato-pallidal circuit Rohleder et al., 2016). Rholeder and colleges showed in their fMRI study that the prelimbic cortex (PrL) was positively correlated with effectiveness of PPI and therefore has an important role in modulation of PPI. Experimental data demonstrating that mPFC lesions remarkably reduced PPI well substantiate that this cortical region plays an important direct role in the regulation of PPI. So far, however, it was practically unknown, how does the neuronal activity of the mPFC modulate PPI. To fulfil one of the major goals of the present experiments, the single neuron discharge characteristics of the mPFC were detected while being just modulated by the startle/pulse alone or PPI/prepulse + pulse stimulation conditions. We have recorded differential mPFC unit responses in relation to the acoustic (i.e. pre-pulse + pulse or pulse alone) stimulations. Based on the results of the present experiments, we determined stimulation dependent distinct groups and subroups of the responsive neurons. Mears (Mears et al., 2006) has observed similar categories of responsiveness in another sensorimotor gating paradigm ("the two tone test") in the mPFC, but he did not use the PPI paradigm. ***The present study provided evidence for the existence of a particular group of mPFC neurons that very selectively responded to prepulse stimulation, thus representing the neural substrate of the PPI.*** After all, in our opinion, in a model organism, such as in the rodent, the PPI is more useful method than the two tone test (Mears et al., 2006), because it is more quantitative and is of more reliable predictive value to identify relevant neural substrates for human neurobiological disorders such as, e.g. for schizophrenia. We found an interesting and certainly important difference from the findings of Mears. In our experiments, two subgroups of the short duration excitatory neurons responding to the prepulse + pulse stimuli were identified: an equal and an unequal one. Neuronal responses of the equal subgroup showed similar activation to both the prepulse and the pulse stimuli during prepulse + pulse trial. In addition to this similarity, the population spike analysis showed similar amplitude and duration of responses during the prepulse + pulse and the pulsealone situation. On the other hand, we recognized distinct features of unit activity during the prepulse + pulse and thepulse alone trial conditions in the unequal subgroup. This neuron population could be further divided into two subdivision: the larger contained the numerous neurons respondingmainly to the pulse stimulus, whereas the smaller primarily changed in activity to the prepulse stimulus. The population spike analysis revealed distinct amplitudes during the prepulse + pulse or the pulse alone stimulus condition, and the duration of the stimulation activated unit activity was shorter during the prepulse + pulse situation than in the pulse alone condition. The neurons of the small, unequal subgroup, with the characteristi-cally prepulse stimulus associated responses, substantiate that the prepulse inhibition is organized, at least in part, at the level of them PFC neurons. It is, therefore, reasonable to suppose that the neurons belonging to this group are playing a distinguished, important role in prepulse inhibition. It is well known, that the PPI has two components: a "hereditary"or non-conditioned component, during which the prepulse stimulus decreases the startle reaction to the pulse stimulus without any learning; and a learned or conditioned component. This latter, so the PPI conditioning, means that the startle reaction can be further decreased with the repetition of prepulse + pulse pairs. Based on these data, we hypothesized that the different patterns (equal or unequal) of single unit activities were caused by differential inputs from various brain areas. Along with this notion it is plausible that in relation to the PPI training the contribution of another learning processes has also to be taken into consideration. This could be the habituational learning component associated to the startle stimulus, which also results in the decrease of startle motor response, but which is independent from the prepulse stimulus (Geyer and Braff, 1987; McIntosh and Gonzalez-Lima, 1991). Important difference between the two learning processes is that the habituation generally occurs with overtraining, while the PPI conditioning is rather typical in an earlier phase of the training (Koch and Schnitzler, 1997; Koch, 1999). The goal of

the present experiments was not, in specific, the investigation of the learning/conditioning processes of PPI but rather the finding of its neuronal correlates and not the habituational component of the training processes. Considering all the aforementioned information, it appears to be reasonable to hypothesize that our new findings concerning the different patterns (i.e.equal or unequal) of unit activites in the short duration PPI group, probably reflect to distinct stages of PPI conditioning. Namely, the neurons activated uneqaully, strongly to the pulse stimulus and weakly to the prepulse stimulus, form the subpopulation which is the most intimately involved in the regulation of PPI, but specifically in the first stage of the conditioning, the effect of the prepulse stimuli on these neurons is pretty small. The second stage of conditioning is demonstrated by the neurons which are reacting equally to the two stimuli, so both the prepulse and the pulse stimuli, can already modulate the activity of these neurons. The third stage is reflected by the small unequal subgroup which showed a relatively large response amplitude to the prepulse stimulus, in contrast to their response to the pulse stimulus which was found to be firmly suppressed. Unfortunately, the protocol followed in the present experiments is not appropriate to confirm these hypotheses: our goal was the mapping of the neuronal activity of the whole mPFC in the pulse startle/pulse alone and PPI/prepulse + pulse trial conditions, so we have moved the electrodes session by session. To detect the conditioning of PPI at the neuronal level of the mPFC, recording of neuronal activity in a constant electrode position across the sessions would be more suitable. We are planning future studies with constant electrode position to confirm this hypothesis. Despite the fact that such equal and unequal neuronal subgroups were not identified in the medium and long duration population, we assume that the neuronal responses of long duration unit activity for the pulse stimulation during prepulse + pulse and pulse alone trial conditions might be associated with motor output, for example with the duration of freezing, whereas the short latency unit activation might be associated with sensory input. Accelerometry is a well-designed method to develop for accurate monitoring of human motion (Lyons et al., 2005; Godfrey et al., 2008; Kavanagh and Menz, 2008). Many of the human diseases or pathological conditions with sympthoms of movement dysfunctions (i.e. Parkinson's disease, stroke, multiple sclero-sis, osteoarthritis, etc.) are accompanied by impaired or greatly reduced mobility. In non-human animal studies, overwhelmingly with rodents, only miniaturizied accelerometers can beused to quantify locomotor behavior in freely moving animals. Head-mounted recording systems can monitor head movements continuously with an on board accelerometer (Ledberg and Robbe, 2011; Gilja et al., 2012; Petyko et al., 2015). A miniature 32-channel head stage amplifier (Noted Bt., Pécs, Hungary) with 3D-accelerometer developed in our laboratory is not only smaller and having various advantageous characteristics compared to other, commercially available equipments in the market but it has also become more accessible to neural signal processing and more smoothly employable in methods for behavioral measurement, such as startle motion tracking. Head postures, such as vertical or horizontal, can be distinguished according to the magnitude of acceleration signals along sensitive axes. However, the single accelerometer approach has difficulty in distinguishing between standing and rearing or other physical activities. Standing and rearing postures can be distinguished by observing different orientations of head axis where multiple accelerometers are attached. In addition to its versatility and its exactness even among very broad scale of circuimtences, the advantages of our accelerometer device include its small size and ability to record data continuously under investigation. Accelerometers also have high resolution at typical sampling frequencies used for head movement detection of freely moving animals. The early component of the acoustic startle has a very short latency. In rats, the shortest latency in the head and neck muscles activation during startle reaction was 5.0–7.5 ms (Pellet, 1990). Previously the early component of the acoustic startle response was usually recorded by electromyography. In our present experiments, for the first time we can report such methodological invention in the literature, measuring the early component, combined with

locomotion recording, have been employed along with a harmonically introduced simultaneous microelectrophyisiological recording technique. The first acceleration wave in our records is assumed to that also measurable by conventional startle measuring systems. We strongly believe that the lower acceleration values measured in the later phase by our apparatus depend on the animal's consolidation response to the fear situation, which definitely can not be observed by using the traditional startle measuring methods. ***The usage of the miniature 3D accelerometer made it possible to quantify head movements for startle stimuli and relate them to behaviorally meaningful head startle and electrophysiological data recorded in freely moving rats.*** We could not find a similar solution in the literature that used accelerometry to explicitly examine startle behavior in freely moving rats.

Conclusion

In the present experiments we have demonstrated that the mPFC neurons respond to startle and PPI stimulation. Single unit recordings revealed three classes of neuronal responses: 1) shortduration group, 2) medium duration group and 3) long duration group. In the short and medium duration groups all responses were excitatory, while in the long duration group both excitatory and inhibitory responses occurred. In the short duration group two sub-groups were also identified: equal and an uneqal one. Results of the present experiments provided evidence for the involvement of mPFC in the control of PPI. In addition, in this paper, we have also demonstrated a new, promising approach of startle reaction measuring with newly designed 3D head accelerometer.

References

Abeles M, Gerstein GL (1988) Detecting spatiotemporal firing patterns among simultaneously recorded single neurons. J Neurophysiol 60:909-924

Alexander GE, Crutcher MD, DeLong MR (1990) Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 85:119-146.

Blumenthal TD, Schicatano EJ, Chapman JG, Norris CM, Ergenzinger ER, Jr. (1996) Prepulse effects on magnitude estimation of startle-eliciting stimuli and startle responses. Percept Psychophys 58:73-80.

Braff DL, Geyer MA (1990) Sensorimotor gating and schizophrenia. Human and animal model studies. Arch Gen Psychiatry 47:181-188.

Braff DL, Geyer MA, Light GA, Sprock J, Perry W, Cadenhead KS, Swerdlow NR (2001) Impact of prepulse characteristics on the detection of sensorimotor gating deficits in schizophrenia. Schizophr Res 49:171-178.

Braff DL, Greenwood TA, Swerdlow NR, Light GA, Schork NJ (2008) Advances in endophenotyping schizophrenia. World Psychiatry 7:11-18.
Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321-352.

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.

Fendt M, Li L, Yeomans JS (2001) Brain stem circuits mediating prepulse inhibition of the startle reflex. Psychopharmacology (Berl) 156:216-224.

Geyer MA, Braff DL (1987) Startle habituation and sensorimotor gating in schizophrenia and related animal models. Schizophr Bull 13:643-668.

Geyer MA, Swerdlow NR, Mansbach RS, Braff DL (1990) Startle response models of sensorimotor gating and habituation deficits in schizophrenia. Brain Res Bull 25:485-498.

Gilja V, Nuyujukian P, Chestek CA, Cunningham JP, Yu BM, Fan JM, Ryu SI, Shenoy KV (2012) A brain machine interface control algorithm designed from a feedback control perspective. Conf Proc IEEE Eng Med Biol Soc 2012:1318-1322.

Godfrey A, Conway R, Meagher D, G OL (2008) Direct measurement of human movement by accelerometry. Med Eng Phys 30:1364-1386.

Graham FK (1975) Presidential Address, 1974. The more or less startling effects of weak prestimulation. Psychophysiology 12:238-248.

Groenewegen HJ (1988) Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. Neuroscience 24:379-431.

Groenewegen HJ, Berendse HW, Wolters JG, Lohman AH (1990) The anatomical relationship of the prefrontal cortex with the striatopallidal system, the thalamus and the amygdala: evidence for a parallel organization. Prog Brain Res 85:95-116; discussion 116-118.

Groenewegen HJ, Wright CI, Uylings HB (1997) The anatomical relationships of the prefrontal cortex with limbic structures and the basal ganglia. J Psychopharmacol 11:99-106.

Gross GW (1979) Simultaneous single unit recording in vitro with a photoetched laser deinsulated gold multimicroelectrode surface. IEEE Trans Biomed Eng 26:273-279.

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.

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.

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.

Huxter J, Burgess N, O'Keefe J (2003) Independent rate and temporal coding in hippocampal pyramidal cells. Nature 425:828-832.

Japha K, Koch M (1999) Picrotoxin in the medial prefrontal cortex impairs sensorimotor gating in rats: reversal by haloperidol. Psychopharmacology (Berl) 144:347-354.

Jay TM, Glowinski J, Thierry AM (1989) Selectivity of the hippocampal projection to the prelimbic area of the prefrontal cortex in the rat. Brain Res 505:337-340.

Kavanagh JJ, Menz HB (2008) Accelerometry: a technique for quantifying movement patterns during walking. Gait Posture 28:1-15.

Kita H (1978) [Inhibition and possible transmitter substance from the frontal cortex to the lateral hypothalamic area in the rat (author's transl)]. Fukuoka Igaku Zasshi 69:223-234.

Koch M (1999) The neurobiology of startle. Prog Neurobiol 59:107-128.

Koch M, Bubser M (1994) Deficient sensorimotor gating after 6-hydroxydopamine lesion of the rat medial prefrontal cortex is reversed by haloperidol. Eur J Neurosci 6:1837-1845.

Koch M, Schnitzler HU (1997) The acoustic startle response in rats--circuits mediating evocation, inhibition and potentiation. Behav Brain Res 89:35-49.

Kodsi MH, Swerdlow NR (1994) Quinolinic acid lesions of the ventral striatum reduce sensorimotor gating of acoustic startle in rats. Brain Res 643:59-65.

Krettek JE, Price JL (1977) The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J Comp Neurol 171:157-191.

Ledberg A, Robbe D (2011) Locomotion-related oscillatory body movements at 6-12 Hz modulate the hippocampal theta rhythm. PLoS One 6:e27575.

Lyons GM, Culhane KM, Hilton D, Grace PA, Lyons D (2005) A description of an accelerometer-based mobility monitoring technique. Med Eng Phys 27:497-504.

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.

McDonald AJ (1987) Somatostatinergic projections from the amygdala to the bed nucleus of the stria terminalis and medial preoptic-hypothalamic region. Neurosci Lett 75:271-277.

McDonald AJ (1991) Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. Neuroscience 44:1-14.

McIntosh AR, Gonzalez-Lima F (1991) Structural modeling of functional neural pathways mapped with 2-deoxyglucose: effects of acoustic startle habituation on the auditory system. Brain Res 547:295-302.

Mears RP, Klein AC, Cromwell HC (2006) Auditory inhibitory gating in medial prefrontal cortex: Single unit and local field potential analysis. Neuroscience 141:47-65.

Nagy B, Szabo I, Papp S, Takacs G, Szalay C, Karadi Z (2012) Glucose-monitoring neurons in the mediodorsal prefrontal cortex. Brain Res 1444:38-44.

Pasquet MO, Tihy M, Gourgeon A, Pompili MN, Godsil BP, Lena C, Dugue GP (2016) Wireless inertial measurement of head kinematics in freely-moving rats. Sci Rep 6:35689.

Pellet J (1990) Neural organization in the brainstem circuit mediating the primary acoustic head startle: an electrophysiological study in the rat. Physiol Behav 48:727-739.

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.

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.

Rohleder C, Wiedermann D, Neumaier B, Drzezga A, Timmermann L, Graf R, Leweke FM, Endepols H (2016) The Functional Networks of Prepulse Inhibition: Neuronal Connectivity Analysis Based on FDG-PET in Awake and Unrestrained Rats. Front Behav Neurosci 10:148.

Schwabe K, Enkel T, Klein S, Schutte M, Koch M (2004) Effects of neonatal lesions of the medial prefrontal cortex on adult rat behaviour. Behav Brain Res 153:21-34.

Schwabe K, Koch M (2004) Role of the medial prefrontal cortex in N-methyl-D-aspartate receptor antagonist induced sensorimotor gating deficit in rats. Neurosci Lett 355:5-8.

Swanson LW (1981) A direct projection from Ammon's horn to prefrontal cortex in the rat. Brain Res 217:150-154.

Swerdlow NR, Braff DL, Geyer MA (2000) Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon. Behav Pharmacol 11:185-204.

Swerdlow NR, Geyer MA (1998) Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. Schizophr Bull 24:285-301.

Szabo I, Czurko A, Csicsvari J, Hirase H, Leinekugel X, Buzsaki G (2001) The application of printed circuit board technology for fabrication of multi-channel micro-drives. J Neurosci Methods 105:105-110.

Thierry AM, Blanc G, Sobel A, Stinus L, Glowinski J (1973) Dopaminergic terminals in the rat cortex. Science 182:499-501.

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, Galosi R, Szabo I, Karadi K, Feldmann A, Peczely L, Kallai V, Karadi Z, Lenard L (2017) Neuronal coding of auditory sensorimotor gating in medial prefrontal cortex. Behav Brain Res 326:200-208.

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.

Uylings HB, van Eden CG (1990) Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. Prog Brain Res 85:31-62.

Varty GB, Higgins GA (1994) Differences between three rat strains in sensitivity to prepulse inhibition of an acoustic startle response: influence of apomorphine and phencyclidine pretreatment. J Psychopharmacol 8:148-156.

Vertes RP (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51:32-58.

Vianna DM, Graeff FG, Brandao ML, Landeira-Fernandez J (2001) Defensive freezing evoked by electrical stimulation of the periaqueductal gray: comparison between dorsolateral and ventrolateral regions. Neuroreport 12:4109-4112.

List of publications

Publications that serve as a basis for the dissertation

Toth A , Petyko Z , Galosi R , Szabo I , Karadi K , Feldmann A , Peczely L , Kallai V , Karadi Z , Lenard L (2017) Neuronal coding of auditory sensorimotor gating in medial prefrontal cortex. Behav Brain Res 326:pp. 200-208.IF: 3,028

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

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

Kállai Veronika , Tóth Attila , Gálosi Rita , Péczely László , Ollmann Tamás , Petykó Zoltán , László Kristóf , Kállai János , Szabó Imre , Karádi Zoltán , Lénárd László (2017) The MAM-E17 schizophrenia rat model: Comprehensive behavioral analysis of pre-pubertal, pubertal and adult rats Behav Brain Res 326:pp. 200-208.IF: 3,028

Lenard L , Ollmann T , Laszlo K , Kovacs A , Galosi R , Kallai V , Toth A , Kertes E , Zagoracz O , Karadi Z , Peczely L Role of D2 dopamine receptors of the ventral pallidum in inhibitory avoidance learning. (2017) Behav Brain Res 326:pp. 200-208.IF: 3,028

Kallai V, Toth A, Galosi R, Szabo I, Petyko Z, Karadi Z, Kallai J, Lenard L (2015) [MAME17 schizophrenia rat model]. Psychiatr Hung 30:4-17.

Szabo I , Mathe K , Toth A , Czurko A (2001) The application of cross-point switch arrays as input selector switch devices for multi-channel electrophysiological experiments. J. Neurosci Methods 111: 75-81. IF: 2,27