069.17 *p187*

HELIX ASPERSA IDENTIFIED NEURONS ON MULTIELECTRODE-ARRAY: ELECTRICAL STIMULATION AND RECORDING

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We have measured spontaneous activity and evoked responses from identified Helix aspersa neurons cultured on multielectrode arrays. Parameters were selected for the best stimulation results by varying adhesion substrates and culture methods. Using whole ganglia, or neurons F1 (and its neighbors from the right parietal ganglion), C1 (MGC), and C3 we measured both beating activity and bursts of action potentials using electrodes localized underneath cell somata.

In vitro cerebral ganglia from 1 to 2 months old Helix aspersa snails have been dissected as described in [1] and placed on multielectrode dishes in modified Leibowitz medium containing either Aplysia hemolymph or a coculture of Helix ganglia.

Multielectrode arrays contain one hundred platinized gold electrodes, which may be individually accessed through contact pads [2]. Acquisition, stimulation, signal processing, and on-line recording are performed by means of virtual instrumentation developed in LabVIEW 5.1 (NI) controlling a data acquisition board (connected to an SCXI stage).

Spontaneous activities ranging from 1 to 10 Hz have been recorded during the first three hours after plating. Stimulation has been done with charge balanced square or sinusoidal pulse trains, with frequencies ranging from 0.5 to 5Hz. Bipolar biphasic single pulses were varied from 100µs to 10ms (per phase) and from 10 to 500nA. Although measure outputs strongly depend on seal impedance between cell and recording electrode, we concluded that multielectrode arrays may be properly used for recordings from Helix aspersa identified neurons.

[1] M.Ghirardi *et al.* (1996), Invertebrate Neuroscience 2: 41-49.

[2] H. Peres *et al.* (1999), Annals of the XIV ICMP99, SP, Brazil: 264.

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069.18 *p188*

NITRIC OXIDE MODULATES RESPIRATORY-LIKE ACTIVITY IN TRANSVERSE BRAINSTEM SLICES OF NEONATAL RATS

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NO production may be linked to EAA synaptic activation within the bulbar respiratory network and may serve as a neuronal messenger especially during hypoxia when there is a massive release of EAA, but its role in the control of breathing is unclear. We tested wether NO modulates respiratory-like activity in brainstem slices. Rhythmic slices (750-850 µm thickness) obtained from anesthetised (ether) neonatal rats (P0-P8) were continuously perfused at 29 °C with artificial CSF bubbled with O_2 (95%) and CO_2 (5%). Drugs were applied in the recording chamber through an additional tank containing the same bubbled aCSF. Respiratory-like activity of the XII nerve rootlet of the slice was recorded through suction electrode, amplified and integrated. SNP was used as NO donor, L-Arginine (L-Arg) was used as a substrate for the NO synthase and D-Arginine (D-Arg) as negative control. NNLA was used to antagonize NOS activity and methylene blue was tested for its effect on cGMP pathway. SNP (10-100 µM) evoked a transient increase in XII burst amplitude which started 1-2 min after beginning of application, reached a maximum effect after 2-4 min and decreased during the remaining time of application (8-10 min) but never below control level. On a same slice, SNP effect was reproduced with L-Arg (40 µM) with a comparable time course, while D-Arg did not evoke any effect. NNLA inhibited the respiratory-like activity as well as methylene blue (50-100 µM). In some cases, SNP and L-Arg slowed the rhythm by increasing the XII burst duration while in other cases frequency was not affected. These results showed that respiratory-like activity *in vitro* is submitted to endogenous modulation by NO and suggest a role for NO during oxygen deprivation.

069.19 *p189*

FOURIER ANALYSIS OF THE FIRING PATTERNS OF NEURONS IN THE RESPIRATORY AND FEEDING NETWORKS OF LYMNAEA STAGNALIS L.

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Fourier-analysis of neuronal firing patterns is an effective technique to detect slight periodicities and correlations in the activity of neural assemblies. In the present study we applied Fourier-methods to classify and quantitatively characterize the firing patterns of molluscan neurons as well as to reveal fine interactions between them. Powersperctra obtained form spike trains of simultaneous intracellular recordings of Lymnaea neurons clearly showed the periodic character of central pattern generating neurons of the cardio-respiratory system and the buccal feeding network. At the same time, further fine oscillations were found not associated with any known CPG rhythms. In the experiments spike arrival times were acquired for each recorded neuron, and the time series were evaluated off-line. The analysis included interspike-interval histograms, spike density functions (SDF) and their Fourier transforms. Apart from the known periodic patterns of the respiratory and feeding CPGs a new oscillation was found in the SDFs of many neurons in the visceral and parietal ganglia. The frequency of this oscillation was found remarkably stable, but varied between 0.2 and 0.4 Hz among preparations. Moderate intracellular hyperpolarizing current injection, low-Ca/high-Mg saline and application of d-tubocurarine failed to abolish this oscillation. Simultaneous recordings of buccal and viscero-parietal neurons did not reveal interactions between the two main CPGs of the Lymnaea in the *in vitro* preparation used.

070.01 *p190*

***c-Fos* AND PEPTIDES IMMUNOREACTIVITIES IN THE CENTRAL EXTENDED AMYGDALA COMPONENTS OF MORPHINE DEPENDANT RATS AFTER NALOXONE PRECIPITATED WITHDRAWAL**

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The central extended amygdala (cEA) is a forebrain macrostructure which may represent a common substrate for acute drug reward and the negative effects of compulsive drug administration on reward function. To test the involvement of cEA components in opiate dependence, we studied the distribution of *c-Fos* immunoreactive neurons, in relation with their neuropeptide content, in brain sections from morphine dependant or naive rats, killed 90 min after naloxone or saline intraperitoneal injection.

Naloxone treatment in naive rats induced *c-Fos* immunoreactivity in the lateral subdivision of the central amygdaloid nucleus, the dorsal part of the lateral bed nucleus of the stria terminalis and the interstitial nucleus of the posterior limb of the anterior commissure. In morphine dependant rats, naloxone injection increased the number of *c-Fos* positive neurons in these structures as well as in other cEA components such as the shell of the accumbens nucleus.

Double immunocytochemical labeling was used to precise the neurochemical nature of *c-Fos* positive neurons in the central amygdaloid nucleus and the lateral bed nucleus of the stria terminalis. While *c-Fos* immunoreactivity was found in CRF-, α-neo-endorphin- or enkephakin-immunoreactive neurons in naive rats after naloxone injection, only a low number of such colocalizations was observed in morphine dependant rats after naloxone injection. This lack of colocalization may reflect a massive release of neuropeptides in the cEA during opiate withdrawal, thus leading to a poor peptides immunoreactivity.

These preliminary results suggest that opiate withdrawal activates specific populations of neurons in the input regions of the cEA and reinforce the concept of extended amygdala as a morphofunctional continuum.

070.02 *p191*

CARBACHOL EVOKED GAMMA ACTIVITY IN MEDIAL ENTORHINAL CORTEX REFLECTS SYNCHRONISATION OF SPATIALLY LOCALIZED SUPERFICIAL INTERNEURONS

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Fast (γ) oscillations in cortex may underlie the rapid co-ordination of large-scale neuronal assemblies in the processing of complex sensory stimuli. *In vivo*, this rhythm is enhanced through cholinergic input from the basal forebrain and is generated *in vitro* with exogenous cholinergic stimulation. Using the isolated whole brain preparation, which combines the advantages of both *in vivo* and *in vitro* conditions, the mechanisms of γ activity in the medial entorhinal cortex (MEC) were studied. Gamma evoked by either arterial perfusion or local MEC injections of carbachol (CCh) was abolished by both systemic and local injections of atropine and bicuculline; the latter manipulations only in spatially restricted zones, directly surrounding the injection sites. Similarly, diffusion of CCh from recording pipettes evoked spatially restricted zones of γ activity. A laminar profile and current source density analysis demonstrated that γ was generated in layer II. Simultaneous multi-site recordings over the surface of the MEC showed a broad distribution of γ, which became less coherent with increased distance between recording sites. This relationship was independent of the amplitude of the signals. In contrast, theta-like oscillations generated by local CCh injections were almost completely coherent, regardless of distance between recording sites. These results suggest that MEC γ reflects the muscarinic synchronisation of superficially located interneurons which give rise to rhythmic IPSPs upon spatially localised "pools" of projection neurons in layer II of the MEC. Since these cells form the bulk of the neocortical input to the hippocampus through the perforant pathway, γ activation *in vivo* may act to "modularise" this input.