БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ • BULGARIAN ACADEMY OF SCIENCES

ПРОБЛЕМИ НА ТЕХНИЧЕСКАТА КИБЕРНЕТИКА И РОБОТИКАТА, **57**
PROBLEMS OF ENGINEERING CYBERNETICS AND ROBOTICS, **57**

София • 2006 • Sofia

Wireless Cell-Recording Methods Applied to Avian Brain Research[*]

Elena Mascalzoni, Lucia Regolin, Ivilin Stoianov

Department of General Psychology, University of Padova, via Venezia 8, 35 100 Padova, Italy
E-mail: Ivilin.Stoianov@unipd.it

The avian brain is considered as an ideal model for the investigation of the biological substrate of behaviour and cognition. Here we first go through some recent data obtained on one of the most widely employed laboratory bird species, the domestic chick. We then outline the nature of the neural signal and describe wireless (telemetric) neural recording methods for studying the neurophysiological bases of behaviour. Finally, we outline few prototypes of miniature telemetry systems allowing neural recording from multiple electrodes.

1. The domestic chicken as a model for studying cognition

In spite of conspicuous differences in sensory systems and adaptations to ecological niches, it seems that most animal species are alike in the overall principles of perceptual structuring and cognition. Moreover, recent research in comparative neuroscience demonstrated a variety of sophisticated cognitive abilities also in non-mammalian species, which could be considered as less different from human beings than previously thought.

The domestic chicken (*Gallus gallus*) is one such species. Chicks' perception, learning and memory abilities, spatial cognition and cerebral and functional asymmetries (lateralization) have been extensively investigated. In fact, chickens showed capable to perceive subjective contours (performing perceptual completion of surfaces not present in the physical distribution of luminance [1]), to use pictorial depth information [2], to perform amodal completion of partly occluded object [3], to perceive stereokinetic illusions [4], to recognize biological motion from a highly impoverished stimulation constructed only from a few moving dots [5], to extract 3D shape from a 2D cast shadow or a point-light-display [6].

---

[*] This research was supported by a joint CNR (Italy) – BAS (Bulgaria) grant 132.48.2.

But there are other reasons for studying cognition in chicks. First of all, they allow us for using diverse behavioural paradigms, such as reinforcement operant conditioning and spontaneous preference following filial imprinting. Moreover, the chick's neuroanatomy and neurophysiology are very well known, allowing for the investigation of the biological bases of cognitive processes. The avian and mammalian telencephalon followed a pattern of convergent evolution, with similar solutions to functional brain organisation. By looking at it, the brain of a 2-day-old chick differs from the mammalian brain mainly because of a not blurred separation between white and grey matter. In spite of this, it has a macroscopic anatomic structure similar to that of the other vertebrates, with cerebellum, optic tectum, and hemispheres easily recognizable (Fig. 1A). Particularly, many brain regions of mammals and birds seem to be homologous, a relevant feature in order to study the philogenesis of behaviour. For instance, it was demonstrated that chickens' visual pathways are equivalent to the mammalian visual pathways. In fact, chicks have two main visual pathways to the telencephalon, the thalamofugal visual system and the tectofugal visual system, considered to be homologous, respectively, to the geniculostriate and to the extra-geniculostriate systems of mammals. Moreover, an important feature of the avian visual system is the complete decussation of the optic nerves at the optic chiasma, enabling us to study hemispheric dominance in different behavioural tasks, through the procedure of monocular exposure/testing.

Using behavioural, lesion and electrophysiological techniques in the domestic chicken and the pigeon as favourite animal models, it was also revealed the fine structure of molecular cascades at the basis of functional synaptic plasticity in a broad range of behavioural paradigms [7].

Therefore, the domestic chick can be considered as an model to study the neurobiology of behaviour. There are many behavioural findings awaiting for an understanding of their neurobiological substrate. One example is, for instance, chicks' ordinal capability.

Numerical ordinal competencies have been demonstrated in various adult non-human animals, such as monkey, rats, salamanders. Rugani and colleagues investigated this ability in very young chicks by training and then testing them pecking either at the 3rd, the 4th or the 6th position in a series of ten identical ones [8]. In all cases chicks at test clearly discriminated the correct ordinal position, while all other positions were chosen at, or even below, chance level.

The results showed that, following training, chicks could accurately identify a given position in a sequence of identical ones. Note that chicks in this experiment could have relied on information other than numerical (ordinal), as they could have used the relational spatial information provided by the experimental apparatus.

These possible alternative explanations were ruled out in a series of control experiments [8], requiring chicks to identify the correct ordinal target when the absolute distance from the starting point at test differs from the training and when both identity and distance of each position changed from trial to trial. Chicks succeeded these generalization tasks.

Young chicks seemed to use ordinality when required to identify a target by its numerical serial position. It seems that the ability to use ordinal information – one routine human ability – is already available in some animal species, very early during ontogeny and may have been selected due to its high adaptive value.

Up to date most of the behavioural findings about cognition in chicks are missing the understanding of the neurobiological substrate of the processes investigated. Concerning complex tasks, there are few investigations on the underlying brain mechanisms for these abilities. The classical behavioural non-invasive techniques achieve only gross structural knowledge. For example, testing chicks in monocular conditions can only unveil the mechanisms at the hemispheric level. Using this method it was suggested that, for example, it is the right hemisphere which is probably involved in numerical ordinal abilities.

Invasive techniques, such as brain lesions and pharmacological inhibitions, are more informative but unfortunately do not look at animals' behaviour in a fully physiological state. It would be much interesting to know more precisely which areas are involved meanwhile the animal is actually performing in various cognitive tasks. This could be made possible with electrophysiological methods that investigate the brain structure by recording neural activity.

2. Neural signal

Information in the neural systems is conveyed by the electrical activity of the neural cells (neurons: see Fig. 1B). The fundamental information transmission unit in the neuron is the action potential (*spike*), a wave of electrical discharge that travels along the membrane of a cell, neural cells especially. Such wave corresponds to a current sink caused by positively charged ions (Na^+) entering the neuron, that in turn corresponds to positive waves generated by the current (mostly K^+) that leaves the cell (see Fig. 1C). Neurons, on average, produce about 5 spikes/sec. with picks up to 100 and minimal inter-spike distance of about 5 ms. [9].

A microelectrode inserted in the brain tissue detects the extracellular electrical activity generated by neurons adjacent to the electrode tip (with respect to a reference electrode). The amplitude and wave shape of the signal depend on a number of factors, including the proximity of the electrode to the neuron, the angle between the electrode surface and the neuron, the neuron geometry, and the particular portion of the neuron

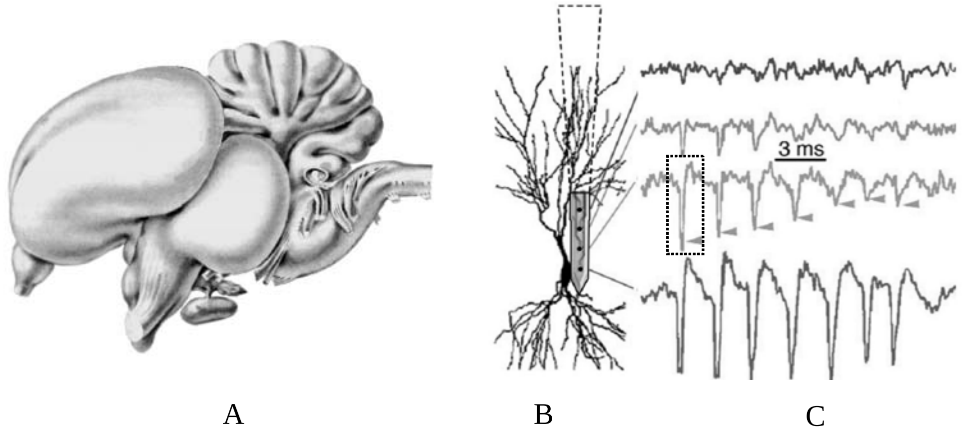

Fig. 1. The typical avian brain gross structure (A); extracellular neural recording with a multi-site (silicon) electrode placed along one neuron (B); a sequence of spikes registered by four recording tips differ by intensity and shape, one spike is outlined (C)

to which the electrode is closest [10] (see Fig. 1C). Despite that spikes produced by multiple neurons could be detected by a same electrode, the spike emitting neurons could be separated on the basis of the characteristics of the spikes (spike sorting), for which purpose about 2.5 ms of the spike waveform is sufficient. Recordings from multiple closely spaced microelectrodes can be used to identify better which of the spikes comes from which cell.

Microelectrodes are typically capacitive, built from polarizable metals, such as tungsten or stainless steel, insulated with teflon or polyimide, and electrolytically sharpened. If the electrode tip is very small (about 1 ?m diameter), the electrode can detect the activity of one neuron (*single-unit recording*). If the tip is larger (25-50 ?m), the electrode could record the activity generated by several nearby neurons (*multi-unit recording*). Neurons close to the electrode produce stronger signal; neurons located beyond some 140 ?m from the electrode tip become indistinguishable from noise [11]. On average one electrode detects two neurons [12]. With electrode impedance of about 1 MΩ (at 1 kHz), the detected signal has an amplitude range from about 20 ?V (noise) peak-to-peak to about 50-400 ?V (spikes) [13], superimposed on a relatively large voltage offset (about 100 mV), and has a bandwidth of 0.3-8 kHz. If the electrode tip is even larger (e.g., 100 ?m) the activity of individual neurons cannot be distinguished and the electrode would record a *field potential* generated by the activity of many cells.

Information processing in the central nervous system is based on single-unit processing, but consists of the interaction of a large number of units [9]. Hence, in order to understand the processing performed in these complex networks of neurons, recording with single electrodes is not sufficient and instead arrays of electrodes are needed.

This has initially been implemented by means of gluing together many single electrodes (e.g., 8-32) and cutoff wire-bundles (microwire bundles) [12]. However, their geometries and reproducibility is limited; they tend to cause considerable insertion damage, and tend to splay out in tissues, making exact recording site uncertain. Nevertheless, they are easily fabricated and therefore are still extensively used for extracellular recording.

A more recent approach uses silicon etching technology to produce electrode arrays with fixed geometry capable of recording from many sites in the biological material simultaneously and with no more damage than a single metal microelectrode [13]. Moreover the signal could be amplified by circuitry directly in the probe, which improves the signal quality. The Michigan electrode array [14], for example, is an array of shanks built upon a 60 ?m-large silicon base and contain from 8 to 16 recording sites (each 10-20 ?m large) on a single shank (see Fig. 1B). The Utah electrode array [15] is a 10×10 3D-matrix of 0.4 mm-spaced silicon electrodes, each of which has a single recording site at its tip and measures: 1.5 mm long; 90/50 ?m base/tip diameter.

3. Wireless cell recording methods

Neural recording in behaving animals is the most direct approach for the analysis of their brain organization and functioning. Physical restraint of the animal subjects is needed when using traditional electrophysiological equipment (e.g., Plexon; Cambridge Electronic Design) in which the microelectrodes are connected with the recording apparatus by means of wires. In experiments with highly mobile animals such as birds, however, it is extremely desirable to have them unrestrained, this could be achieved by means of wireless communication.

Wireless transmission of bioelectric data (*telemetry*), such as electrocardiograms or electroencephalograms has been used in the biomedical sciences for several decades [16]. However, wireless broadcasting the neural activity of small animals has to meet more specific demands: the remote module should be miniature and light (e.g., max. 10-15 cm^2; 10-20 g), have an autonomy of a few hours, and simultaneously transmit the signal from multiple electrodes.

The neural signal can be transmitted via radio frequency (RF) using either analogue or digital encoding. Multiplexing a large number of analogue channels for further RF transmission is impractical. Two channels have been transmitted using stereo FM modulation [17, 18]. One potential obstacle for this approach is interference with radio broadcasting. Hence, the general solution for wireless multi-channel telemetry involves digital coding [13].

The critical component in digital telemetry is the remote unit. It consists of: (i) *Headstage*: A circuitry attached immediately to the electrode that reduces the large input impedance and pre-amplifies the signal; if large gain is used, the signal should be filtered (see ii) in order to avoid saturation. (ii) *Amplifier* with variable gain, it filters the signal 0.2-10 kHz and amplifies it about 10 000 times, in order to obtain a range of about 3 V; (iii) *Analog-Digital Converter*: Samples each channel with at least 10 kHz, 10-bits precision (i.e., 100 kbs per channel); (iv) *Microcontroller* with a microprocessor and buffer memory that controls the whole process: Sampling, buffering, compression, and transmission; (v) *RF-transmitter*: Sends the data via radio waves; (vi) B*attery*: It should be physically small and allow few hours of operation.

Sampling digital signal from multiple electrodes might produce excessive raw data. For example, 100 electrodes produce about 10 Mbs data rate. However, assuming that on average one electrode detects two neurons, each of which generates 10 spikes per second, and that a 2.5 ms waveform segment of 8-bit samples is sufficient for spike discrimination, transmitting only this signal results in an almost costless compression that reduces the data rate about 25 times (4 kbits per electrode). Sophisticated compression could reduce the data rate even more, but it would also require more computational and energy resources.

The RF-transmission could be custom-designed or based upon established wireless protocols, such as WiFi, Bluetooth, or Zigbee [19]. It should allow a range a few meters (application-dependent) and provide sufficiently high data transfer rate (e.g., 1 Mbits/s). Custom solutions might allow tiny hardware implementations, but require complex tuning. Standard wireless protocols, instead, have low-cost single-chip implementations that are easy to embed and set-up.

4. Miniature telemetry systems

The complete implementation of a digital remote unit could be too large for a small animal. For example, Obeid et al. [20] developed and tested *in-vivo* (on monkey) a 16-channel telemetry system based upon Wi-Fi communication that weights as much as 240 g. Nevertheless, ongoing technological developments allow the development of much smaller systems. At present, however, to our knowledge neural recordings with truly miniature multi-channel telemetry systems that could be applied to avian brain research have not been reported (with the exception of 2-chanel analogue transmission [17]).

The smallest digital telemetry unit reportedly tested *in vivo* weights more than 26 g and is 5.4 ×1.2 cm large [21]. It uses IR communication that features relatively low bandwidth (57.6 kbits), hence it could transmit (and store) only decoded spike information (and a small proportion of the raw data for off-line analysis). The remote unit is based upon CY8C27443 Programmable System-on-a-Chip (by Cypress Semiconductor). Although that system was projected to record from 12 electrodes, the microcontroller could computationally handle only one channel. Hence, this system is not suitable for multi-channel recordings.

Geortchev et al. proposed a prototype of a telemetry system designed specifically for avian brain research [19, 22]. It is based upon ATmega128L microcontroller (by Atmel) and Promi ESD Bluetooth communication module (by Initium). The remote unit consists of three stacked boards (amplifier, microcontroller, and Bluetooth) and is 3 × 3 × 1.5 cm large; weights about 25 g, and consumes about 100 mW. A button-size 3.3 V lithium battery allows 4 hours of operation. Up to eight electrodes could be sampled by eight A/D converters (10 kHz; 10 bits) integrated in the microcontroller. More electrodes could be sampled by an additional A/D converter. The unit is controlled by custom software that samples the data, detects spikes (with individual thresholds for each channel) and transmits spike waveforms (4 ms, 40 samples per spike). The 720 kbits Bluetooth bandwidth is sufficient for the transmission of sustained neural activity at all electrodes. The data is received by a base module that further communicates it to a PC via a USB port. One useful feature of this module is that it also reconstructs the analogue waveforms of up to two channels, which is useful for simple cable replacement and facilitates the system set-up.

A similar approach for miniature telemetry was more recently proposed by Cieslewski et al. [23]. It's based upon MSP430/F1611 microcontroller (by Texas Instruments) and nRF2401 RF-transmitter (by Nordic). The remote unit is 3.5 ×7 cm large. The microcontroller features eight 12-bit A/D converters. The data is sampled with 20 kHz, but spike waveforms are transmitted only. When operating in a low-consumption mode (105 mW overall unit consumption), the Nordic RF-transmitter has 240 kbitss bandwidth, which could be extended up to 1 Mbs if operated in full-range mode (with increased energy consumption). The data is received by a base-unit implemented with microcontroller and transceiver as those of the remote unit, which passes it then to a PC via a USB port.

Another promising telemetry solution has recently appeared as a commercial product [24], but is no more available as such. It uses Bluetooth communication and could transmit data from up to 16 electrodes. The remote unit is 5 ×5 × 0.6 cm large, weights between 35 and 50 g, depending on battery, which in turn allows up to 8 hours of operation. The unit digitalizes the data, detects spikes (based upon channel-specific thresholds), and transmits spike waveforms only. A normal Bluetooth transmitter attached to a PC receives the data and passes it to a custom software for further analysis.

5. Conclusions

Recent data have been reported employing the domestic chick as an animal model for the investigation of the neurobiological bases of behaviour and cognition. This avian species has in fact been shown to display rather sophisticated cognitive skills, moreover, its neuro-anatomy is very well known, particularly for what concerns areas homologous to those of the mammalian brain [25]. Investigation of complex behaviour in this as well as other animal models should be done by neural recordings from multiple sites in physically unconstrained animals, using wireless data transmission (telemetry). Telemetry has been used for years, but employing systems which either permit recording from just few microelectrodes or are physically too large and heavy for small laboratory animals. We identified two recent prototypes of miniature systems recording from multiple electrodes [19, 23] and one commercially product [24]. However, *in-vivo* experimentations should still prove the functionality of those systems and give clues about their improvements.

References

1. Zanforlin, M. Vizual Perception of Complex Forms (Anomalous Surfaces) in Chicks. – Italian Journal of Psychology, Vol. **8(1)**, 1981, 1-16.
2. Forkman, B., G. Vallortigara. Minimization of Modal Contours: an Essential Cross-Species Strategy in Disambiguating Relative Depth. – Animal Cognition, Vol. **2**, 1999, 181-185.
3. Regolin, L., F. Marconato, G. Vallortigara. Hemispheric Differences in the Recognition of Partly Occluded Objects by Newly Hatched Domestic Chicks (Gallus Gallus). – Anim Cog., Vol. **7**, 2004, 162-170.
4. Clara, E., L. Regolin, M. Zanforlin, G. Vallortigara. Domestic Chicks Perceive Stereokinetic Illusions. – Perception, Vol. **35**, 2006, 983-992.
5. Regolin, L., L. Tommasi, G. Vallortigara. Visual Perception of Biological Motion in Newly Hatched Chicks as Revealed by an Imprinting Procedure. – Animal Cognition, Vol. **3**, 2000, 53-60.
6. Mascalzoni, E., L. Regolin, G. Vallortigara. Perception of Impoverished Displays in an Animal Model. – Journal of Experimental Psychology: Animal Behaviour Processes, 2006 (in preparation).
7. Rose, S. Memory beyond the synapse. – Neuron Glia Biology, Vol. **1**, 2004, 211-217.
8. Rugani, R., L. Regolin, G. Vallortigara. Rudimental Numerical Competence in 5-Day-Old Chicks: Identification of Ordinal Position. – Journal of Experimental Psychology: Animal Behaviour Processes, 2007 (in press).
9. Nadasdy, Z. Spike Sequences and Their Consequences. – J. Physiol (Paris), Vol. **94**, 2000, 505-524.
10. Lewicki, M. S. A Review of Methods for Spike Sorting: The Detection and Classification of Neural Action Potentials. – Network, Vol. **9**, 1998, R53-78.
11. Henze, D. A., Z. Borhegyi, J. Csicsvari, A. Mamiya, K. D. Harris, G. Buzsaki. Intracellular Features Predicted by Extracellular Recordings in the Hippocampus in Vivo. – J. Neurophysiol, Vol. **84**, 2000, 390-400.
12. Nicolelis, M. A., A. A. Ghazanfar, B. M. Faggin, S. Votaw, L. M. Oliveira. Reconstructing the Engram: Simultaneous, Multisite, Many Single Neuron Recordings. – Neuron, Vol. **18**, 1997, 529-537.
13. Wise, K. D., D. J. Anderson, J. F. Hetke, D. R. Kipke, K. Najafi. Wireless Implantable Microsystems: High-Density Electronic Interfaces to the Nervous System. – In: Proc. of the IEEE, Vol. **92**, 2004.
14. Najafi, K., K. Wise. An Implantable Multielectrode Array with On-Chip Signal Processing. – IEEE J. of Solid-State Circuits, Vol. **21**, 1986, 1035-1044.

15. Nordhausen, C. T., E. M. Maynard, R. A. Normann. Single Unit Recording Capabilities of a 100 Microelectrode Array. – Brain Res, Vol. **726**, 1996, 129-140.
16. Kimmich, H., J. Vos. Biotelemetry. Leiden. Meander, 1972.
17. Nieder, A. Miniature Stereo Radio Transmitter for Simultaneous Recording of Multiple Single-Neuron Signals from Behaving Owls. – J. Neurosci Methods, Vol. **101**, 2000, 157-64.
18. Chien, C. N., F. S. Jaw. Miniature Telemetry System for the Recording of Action and Field Potentials. – J. Neurosci Methods, Vol. **147**, 2005, 68-73.
19. Geortchev, V., I. Stoianov, R. Krasteva, A. Boneva, D. Batchvarov, K. Stanishev, R. Zahariev, G. Vallortigara. Digital Communication for Telemetric Multi-Neuron Recordings. – Academic Open Internet Journal, 2005.
20. Obeid, I., M. A. Nicolelis, P. D. Wolf. A Multichannel Telemetry System for Single Unit Neural Recordings. – J. Neurosci Methods, Vol. **133**, 2004, 33-8.
21. Mavoori, J., A. Jackson, C. Diorio, E. Fetz. An Autonomous Implantable Computer for Neural Recording and Stimulation in Unrestrained Primates. – J. Neurosci Methods, Vol. **148**, 2005, 71-77.
22. Geortchev, V., I. Stoianov, D. Batchvarov, R. Krasteva, A. Boneva, K. Stanishev, G. Vallortigara. Telemetry System for Single-Cell Recording in Birds: Bluetooth-Based Digital Communication. – In: XV Int. Conf. Robotics and Mechatronics-2005, Drjanovo monastery, Bulgaria, 2005.
23. Cieslewski, G., D. Cheney, K. Gugel, J. C. Sanchez, J. C. Principe. Neural Signal Sampling via the Low Power Wireless Pico System. – In: EEE Int. Conf. of the Eng. in Medicine and Biology Society, New York, 2006.
24. TBSI. Wireless Backpack System Description, 2006.
25. A. B. N. Consortium”. Avian Brains and a New Understanding of Vertebrate Brain Evolution. – Nature Reviews in Neuroscience, Vol. **6**, 2005, 151-158.

Безпроводниковые методы записи невронной активности и их приложения при исследовании мозга птиц

Елена Маскалцони, Лючия Реголин, Ивилин Стоянов

Университет Падова, Факультет общей психологии, ул. Венеция 8, 35100 Падуа, Италия
E-mail: Ivilin.Stoianov@unipd.it

(Резюме)

Птичий мозг – один из самых подходящих моделей при изследовании биологической основы поведения и восприятия. В статье в первую очередь представлены некоторые реультаты последных исследований одной из самых использованных лабораторных птиц – домашней курицы. Затем описан характер невронного сигнала и некоторые телеметрические методы его записи, приложимые при неврологических исследованиях поведения птиц. В заключении описаны несколько прототипов миниатюрных телеметрических систем записи невронной активности, регистрированных с многоканальным датчиком.