P122

Dopamine neurons of the periaqueductal grey participate in nociceptive responses after opiates

Juan A. Flores, Emilio Fernandez Espejo, Fadwa El Banoua, Beatriz Galan and Isabel Caraballo

Departamento de Fisiologia Medica, Universidad de Sevilla, 41009 Sevilla, Spain

The dopaminergic network of the periaqueductal grey (PAG) was described several years ago (Lindvall & Björklund, 1974). The periaqueductal grey is a critical locus controlling nociceptive responses, as well as opiate-induced analgesia. However, the role of dopaminergic PAG neurons on nociceptive responses is not known. The objectives of the study were 2-fold: (i) to discern if the dopamine neurons of the PAG are activated after opiate administration, and (ii) to establish the effects of selective lesions of the DA neurons of the PAG on opiate-induced analgesia in rats. Nociceptive threshold was evaluated through the tail-immersion (for evaluating a spinal reflex) and the hot-plate tests (which allows evaluation of more integrated pain-related responses), establishing a short cut-off time (25 s) to further minimize animal suffering. At the end of the experiments the animals were humanely killed. The findings revealed that the dopaminergic cells of the PAG were activated following opiate treatment, since they expressed c-Fos after heroin (500 and 1000 μg kg^{-1} S.C.) and morphine (400 and 800 μg kg^{-1} S.C.), as evaluated through immunohistochemistry (in comparison with saline-treated rats). Following dopamine depletion of the mesencephalic periaqueductal grey (52.7 % dopamine cell loss, 80.7 % reduction of *in vitro* dopaminergic peak as measured by voltammetry), the dose–response curve to opiates was shifted to the right in the hot plate test, and analgesia was significantly attenuated ($P < 0.01$, 2-way ANOVA and *post hoc* Newman-Keuls test). The present study provides evidence that the dopaminergic network of the periaqueductal grey is activated after opiate treatment, and mediates integrative nociceptive responses since dopamine loss attenuates opiate-induced analgesia in the hot plate test.

Lindvall O & Björklund A (1974). In *Hanbook of Psychopharmacology*, ed. Iversen L *et al.* Plenum Press, New York.

This study was supported by Spanish Ministerio de Educacion y Cultura (PM98-015), Plan Andaluz de Investigacion (CVI-127) and Laboratorios Dr Esteve (Barcelona, Spain).

All procedures accord with current local guidelines.

P123

Dopamine neurons of the periaqueductal grey control heroin-induced reward

Emilio Fernandez Espejo, Juan Antonio Flores, Fadwa El Banoua, Beatriz Galan and Isabel Caraballo

Departamento de Fisiologia Medica, Universidad de Sevilla, 41009 Sevilla, Spain

The dopaminergic network of the periaqueductal grey was described several years ago (Lindvall & Björklund, 1974). However, considering the key role of dopamine in opiate addiction, it is surprising that the dopaminergic network of the periaqueductal grey area has been ignored in drug reinforcing studies. The objectives of the study were threefold: (i) to further describe the morphological characteristics of the mesencephalic part of the dopaminergic network of the periaqueductal grey (mPAG) in rats, (ii) to discern if these dopaminergic cells are activated after heroin administration (500 μg kg^{-1} S.C.), and (iii) to establish the effects of selective lesions of the DA neurons of the mPAG on heroin-induced reward (as measured through conditioned place preference) and locomotor sensitization. At the end of the experiments the animals were humanely killed.

The mesencephalic periaqueductal grey network was observed to be composed of three types of dopaminergic cells putting out fibres mostly running in the periaqueductal grey, after immunohistochemistry. These dopaminergic cells were activated following drug treatment, since they expressed c-Fos after heroin (in comparison with saline-treated rats). Finally, following dopamine depletion of the mPAG (52.7 % dopamine cell loss through cell counting, 80.7 % reduction of *in vitro* dopaminergic peak as measured through voltammetry), conditioned place preference to heroin was abolished in lesioned rats ($P < 0.05$, Wilcoxon test), but not heroin-induced sensitization.

The present study provides evidence that the dopaminergic network of the mesencephalic periaqueductal grey is activated by heroin and controls the rewarding effects of this opiate drug. This dopaminergic system should be included as critical for opiate-induced reinforcement, apart from the mesocorticolimbic system.

Lindvall O & Björklund A (1974). In *Hanbook of Psychopharmacology*, ed. Iversen L *et al.* Plenum Press, New York.

This study was supported by Spanish Ministerio de Educacion y Cultura (PM98-015), and Plan Andaluz de Investigacion (CVI-127).

All procedures accord with current National and local guidelines.

P124

Effect of nucleus paragigantocellularis latralis lesion on the conditioned place preference in the presence and absence of clonidine

H. Fathi-Moghadam, M. Kesmati and H. Mohammad Pour Kargar

Department of Physiology, School of Medicine, Ahwaz University of Medical Sciences, Ahwaz, Iran

The nucleus paragigantocellularis latralis (LPGi) is located in the rostral ventrolateral medulla (RVLM), a brainstem region that regulates homeostatic functions such as blood pressure and cardiovascular reflexes, respiration, pain and opiate withdrawal syndrome. LPGi has many anatomical relationships with important nuclei such as periaqueductal gray (PAG), locus coeruleus (LC), dentate and raphe. In the present study we have examined the role of LPGi in the conditioned place preference (CPP) induced by morphine in the presence and absence of clonidine in the rat.

We used 45 male N-MRI rats. Animals were divided into seven groups: (1) control; (2) control + saline; (3) sham; (4) LPGi lesion; (5) lesion + 0.02 mg kg^{-1} clonidine; (6) lesion + 0.2 mg kg^{-1} clonidine; and (7) lesion + 2 mg kg^{-1} clonidine. LPGi nucleus was destroyed bilaterally by electrical lesion under ketamine (100 mg kg^{-1}) and Rampone (3 mg kg^{-1}), I.P., anaesthesia. Data were analysed using Student's paired *t* test. After the recovery period, CPP was induced by Hand's method (Hand *et al.* 1989). One hour before testing, clonidine was administered (I.P.).

We did not find any significant differences between the results of control, control + saline and sham groups in the CPP test. There was a significant increase in the CPP between sham and

lesion + saline groups ($P < 0.019$). There was a significant decrease between lesion + saline and lesion + clonidine (0.02, 0.2 and 2 mg kg^{-1}) groups ($P < 0.002$) in the CPP test.

LC is an important noradrenergic nucleus in the brain and is involved in memory, pain and anxiety. LPGi sends its major excitatory projections to the LC. Lesion of LPGi decreases the activity of LC. It seems that increase of CPP in the LPGi lesioned group is related to the decrease of LC activity. LPGi plays a modulatory role in the CPP. In the intact animals, clonidine did not alter CPP at all, but it decreased CPP significantly ($P < 0.002$) in all the lesioned groups. Our results indicate that LPGi lesion induced α_2 adrenergic sensitivity in the LC and follower centres.

Hand TH *et al.* (1989). *Psychopharmacology* **98**, 61–67.

Mohammad pour Kargar was a MSc Student at the Ahwaz College of Science.

All procedures accord with current National and local guidelines.

P125

Paradoxical dose-dependent action of pilocarpine on sleep in rats

M.A. Comas*, A. Gamundi*, A.M.L. Coenen†, G. Van Luijtelaar†, S. Tejada* and R.V. Rial*

**Laboratori de Fisiologia, Universitat de les Illes Balears, Palma de Mallorca, Spain and †Department of Biological Psychology/NICI, Katholieke Universiteit Nijmegen, The Netherlands*

Cholinergic systems play an important role in generating EEG as well as in regulating the vigilance states. Classically, the muscarinic cholinergic agonists (i.e. arecholine, RS86, pilocarpine, etc.) shortened REM latency, increased REM time and reduced slow wave sleep (SWS). These results led to the proposal of a cholinergic control for REM.

Pilocarpine is a partial cholinergic agonist with a moderate affinity for M1 muscarinic receptors and higher for M5 ones. Its affinity for M2 receptors, which are believed to play a role in REM induction, is of the same order as that of arecoline and RS86. Rapid REM induction was achieved with a high arecholine dose, but dose-response studies for pilocarpine REM sleep induction are lacking. This study aims at analysing the effects of pilocarpine on sleep architecture in rats.

Nine male Wistar rats weighing 410 ± 10 g (S.E.M.) were used. Under isoflurane anaesthesia, each rat was implanted with two epidural cortical electrodes (AP +2.0, ML +2.5 and AP −6.0, ML +4.0), and a reference one over the cerebellum. Two additional electrodes were used for neck EMG recording. After recovery from surgery, bipolar EEG and EMG were recorded during dark time in freely moving animals for 120 min, starting 30 min after pilocarpine injection. Recordings were made in three randomly ordered conditions: I.P. saline and pilocarpine at both 1 mg kg^{-1} and 3 mg kg^{-1}. Latencies from injection time and duration of (1) active waking, (2) SWS, (3) REM and (4) theta rhythm periods, were calculated. The study was performed in accordance with the guidelines of the local ethical committee for animal studies. After the experiments, the animals were killed by an overdose of barbiturate anaesthetic.

Significant differences in duration of the behavioral states between saline and low dose pilocarpine were not found. On the contrary, the latencies increased with the low dose for REM (ANOVA, $P < 0.05$) and caused an almost total REM loss with the high dose. For SWS, latencies increased from 570 ± 60 to 694 ± 75 and 5400 ± 1300 s for saline, low and high doses respectively (means ± S.E.M., $P < 0.0001$). As the increase in latencies was so large, the total amount of the two phases was significantly reduced ($P < 0.001$ for SWS and $P < 0.05$ for REM). Instead, the theta rhythm time was increased for every dosage ($P < 0.0003$), remaining without change the active waking.

In conclusion, the effect of pilocarpine in rat sleep is a strong reduction in both SWS and REM and a high increase in theta rhythm time. However, these results contradict previous ones where the activation of cholinergic systems greatly increased REM duration and decreased its latency.

All procedures accord with current National and local guidelines.

P126

Effect of sympathetic neurotransmitter on cytokine (IL-2 and TNFα) release and lymphoproliferation of peritoneal leucocytes from adult, old and very old BALB/C mice

M. Puerto, N. Guayerba and M. De la Fuente

Department of Animal Physiology, Faculty of Biological Sciences, Complutense University, Madrid, Spain

There is increasing evidence of bidirectional communication between the nervous and the immune systems, but the age-related changes in this communication, including the effects of neurotransmitters on immune cells, have been scarcely studied. In the present work the *in vitro* effects, on several functions of leucocytes from adult (22 ± 2 weeks old), old (72 ± 2 weeks old) and very old (128 ± 2 weeks old) mice, of two neurotransmitter of the sympathetic nervous system, noradrenaline (NA) and neuropeptide Y (NPY), individually and jointly have been studied.

The concentrations of neurotransmitters used were: 10^{-12}, 10^{-10} and 10^{-8} M for NPY and 10^{-12}, 10^{-10}, 10^{-8} and 10^{-6} M for NA as well as these concentrations of NA plus 10^{-10} M of NPY. Peritoneal leucocytes from BALB/c mice were obtained without killing of animals. Lymphoproliferation both spontaneous and in response to mitogens (concanavalin A (conA) and lypopolysaccharide (LPS)), and the release of cytokines such as interleukin 2 (IL-2) (measured in cultures with conA) and tumour necrosis factor α (TNFα) (measured in cultures with LPS), were the functions studied.

The results show a stimulatory effect of neurotransmitter on spontaneous lymphoproliferation at all ages studied. Lymphoproliferation in response to mitogens was lower in old than in adult mice. The proliferation of lymphocytes in the presence of ConA was inhibited, not influenced and stimulated by NPY and NA in cells from adult, old and very old mice, respectively. The same results were obtained for IL-2. The lymphoproliferation in response to LPS was stimulated, not affected and inhibited by the neurotransmitter in cells from adult, old and very old mice, respectively. The TNFα release was inhibited at all ages studied, but with all concentrations of neurotransmitter only in adult mice. Always, the effects shown in the joint presence of NPY and NA were more related to those shown with NA. These results suggest a modulatory role of sympathetic neurotransmitters such as NA and NPY, which changes with ageing.

The present work was supported by a FISS (99/0815) grant.

All procedures accord with current National and local guidelines.

P127

Anti-inflammatory pretreatment protects against self-stimulation impairment produced by 6-hydroxydopamine lesions in the rat

F. Vives, I. Rivas, I. Prieto, M. Ramirez, M. Arauzo, B. Morales, J.M.R. Ferrer and F. Alba

Department of Physiology and Department of Biochemistry, Faculty of Medicine, University of Granada, Granada, Spain

The etiology of Parkinson's disease is multifactorial. Genetic, environmental and inflammatory processes are involved. Recently, it has been shown that anti-inflammatories reduce lesion volume after postnatal excitotoxic damage (Acarin *et al.* 2002). If inflammatory response is related to the progression of secondary neuronal damage, pretreatment with anti-inflammatories may improve some Parkinson's disease (PD) symptoms. PD is characterized by a severe decrease of brain dopamine (DA) and brain DA dysfunction impairs intracranial self-stimulation (ICSS). The objective of this research was to assess if two anti-inflammatories (indomethacin and chloroquine) protect against experimental PD produced by 6-hydroxydopamine (6-OHDA) lesions.

Thirty rats were divided into four groups: a control group (sham operated, $n = 10$), which received vehicle injection into right striatum; a lesion group ($n = 9$) which received 6-OHDA injection into right striatum; an indomethacin group, which after 5 days of treatment with indomethacin (2 mg in DMSO per kg body weight) was lesioned with 6-OHDA; and a fourth group, which received 5 days of chloroquine (5 mg kg^{-1} b.w.) before 6-OHDA lesion. Rats were anaesthetized with Equithensin (2 ml (kg body weight)$^{-1}$ s.c. Co-ordinates of lesion were: 0 mm AP, 3 mm lateral and 5 mm deep. On the last day of pretreatment, they were anaesthetized with equithensin and 4 μl of saline or 6-OHDA (32 μg/4 μl of saline) was injected stereotaxically. Two months after the lesion, rats were implanted bilaterally with monopolar electrodes into the medial prefrontal cortex. Then, they were trained to press a bar to receive electrical ICSS. All of them learnt ICSS behaviour in 1 week, and current intensity was manipulated in order to obtain the lowest intensity which would generate an optimal rate of response for a particular animal. This current intensity was used to establish a reliable ICSS performance. Spontaneous motor activity was also measured as a control. Animals were killed humanely.

The lesion group showed a significantly lower ICSS rate than groups with anti-inflammatory pretreatment and 6-OHDA lesions, which, in turn, showed a lower rate than control group. Significant differences in spontaneous motor activity were not found. In conclusion, the administration of indomethacin and chloroquine protected against 6-OHDA effects on prefrontal self-stimulation.

Acarin *et al.* (2002). *Stroke* **33**, 2499.

Junta de Andalucia through PAI CTS-438 and PAI CVI-221 (Acciones Coordinadas) supported this work.

All procedures accord with current National and local guidelines.

P128

Variable motor asymmetry in rats

Ll. Gené, M. Akaârir, S. Aparicio, C. Garau, C. Roca and M.C. Nicolau

Laboratori de Fisiologia, Universitat de les Illes Balears, Palma de Mallorca, Spain

Motor asymmetries have been recognized in animals and human beings. They are caused by a differential development of some brain areas, especially the nigrostriatal dopaminergic system. The anatomical and functional differences between the two brain sides cause a consistent motor asymmetry in rats, which is easily quantified by using a rotating behaviour test. The results of this test are considered as defining the degree of asymmetry for a given animal, with results classified as having either low or high asymmetry but also as showing either right or left rotating preference. This classification is considered to remain constant for each individual. However, this is normally achieved after a relatively short study, with the animals placed in the rotometer for only a few hours.

On the other hand, there are results showing that the handedness in human beings can change in relation to the activity states, with a particular subject showing right hand dominance during day time (as measured by bilateral wrist actimetry), while this can change during sleep. It has also been shown that there are bilateral differences in the circadian activity of the suprachiasmatic nucleus, reaching a phase shift even of over 100 min between the two sides. These results are of interest because they can provide a basis to explain the extreme asymmetry found during the sleep of aquatic animals.

The present report aims at measuring the rotating asymmetry of rats during a relatively long time span (over 48 h). This should be enough to give indications on whether the side preference is constant for a given animal or, alternatively, the preference changes along the day.

Eight Wistar male rats weighing between 250–400 g were placed in a computerized rotometer consisting of a 30 cm diameter circular arena. The rotations of the animals were fed to a computer which stored both the movements and the time in which each movement took place. Commercial food pellets and tap water were freely available in the rotating chamber during the tests. The study was performed under approval of the Ethical Committee of the University of Balearic Islands for animal experimentation.

As a result, five animals showed sustained epochs with shifted side preference, covering at least 30 % of the recorded time. In most cases, the preference shift occurred within 4 h after lights off. The remaining three animals showed a constant asymmetry towards one side only. This suggests that the shifts in side prefference for rotation are related to the circadian rhythm.

As a conclusion, it has been found that the side preference of a high proportion of rats suffers important changes when measured during an extended period of time.

This study was supported by FIS grant 00/0022-01.

All procedures accord with current National and local guidelines.

P129

Nitric oxide modulation of motor behaviour in swiss mice

E.A. Del Bel*, F.S. Guimarães†, L.P. Nucci-da-Silva*, M. Lazzarini*‡ and V. Tumas§

*Departments of *Physiology, FORP, †Pharmacology, ‡Physiology and §Neurology, FMRP, Campus USP, Ribairao Preto, Sao Paulo, 14149-900 Brazil*

Nitric oxide (NO) has been proposed as a new neurotransmitter/neuromodulator in the central nervous system (CNS). In the present experiments we have examined the effects of inhibitors of NO synthase (NOS), the enzyme responsible for NO formation, on some mouse behaviour tests. In previous experiments, systemic injections of low doses of NOS inhibitors, induced anxiolytic effects in the elevated plus maze, whereas higher doses decrease maze exploration (Lino de Oliveira *et al.* 1997). This latter effect may involve motor effects of this compounds.

Male albino-Swiss mice (25–30 g) were housed in groups, in a temperature-controlled room with a 12 h light–dark cycle, with free access to food and water. The acute effects of the NOS inhibitors N^G-nitro-L-arginine (L-NOARG, 10–80 mg kg^{-1} I.P.) and 7-nitroindazole (7-NIO, 3–30 mg kg^{-1} I.P.) on exploratory activity were analysed in an open field arena. Drug effects on catalepsy were examined in the hanging-bar and wire-ring test. Footprint pattern after treatment with the two NOS inhibitors was evaluated and the results compared with those obtained with the dopamine D2 receptor antagonist haloperidol (1–2 mg kg^{-1} I.P.). Sub-chronic (twice a day for 4 days) effects of L-NOARG (40 mg kg^{-1} I.P.) or 7-NIO (30 mg kg^{-1}, I.P.) were also tested in the open field arena and catalepsy test. The experiments were carried out according to the Brazilian Society of Neuroscience and Behaviour guidelines for care and use of Laboratory animals. The animals were killed humanely.

L-NOARG and 7-NIO decreased locomotion and rearing in the open field arena. Both drugs induced catalepsy in the hanging-bar test but did not change footprint pattern. The cataleptic effect of L-NOARG in the hanging bar and wire-ring tests were highly correlated ($r = 0.927$). Acute administration of L-NOARG had an additive effect with haloperidol (dopamine D2-receptor inhibitor) and was potentiated by pre-treatment with WAY 100135 (a 5-HT1 receptor antagonist), ketanserin (5HT2a and α-adrenergic receptor antagonist) and amantadine (glutamatergic receptor antagonist). Pre-treatment with atropine sulphate or biperideno (muscarinic receptor antagonists) and apomorphine (D2 receptor blocker) inhibited L-NOARG-induced catalepsy. The exploratory and cataleptic effects of L-NOARG and 7-NIO provided evidence for tolerance after sub-chronic treatment. Neither drug changed footprint pattern. Sub-chronic L-NOARG treatment induced cross-tolerance to haloperidol catalepsy and an increase of NADPH-diaphorase neurons in the dorsal part of caudate, acumbens and segmental pedunculus pontinus nucleus (Del Bel & Guimaraes, 2000). There was a decrease in NADPH-diaphorase neurons in the substantia nigra compacta.

The results confirm that inhibition of neuronal NO formation induces an impairment of exploratory and motor behaviour and give support to the hypothesis that NO plays a role in motor behaviour control, probably modulating dopaminergic, serotonergic and cholinergic neurotransmission. This effect does not seem to involve aspects evaluated by footprint analysis, such as weight support, trunk stability and foot placement.

Del Bel EA & Guimãraes FS (2000). *Psychopharmacology* **147**, 356–361.

Lino de Oliveira S *et al.* (1997). *Pharmacol Biochem Behav,* **56**, 55–59.

This work was supported by FAPESP and CNPQ.

All procedures accord with current local guidelines.

P130

Interhemispheric EEG dependences and propofol anaesthesia in human subjects

A. Gamundi*, M.A. Comas*, S. Tejada*, C. Roca*, E. Pereda†, J. González‡, CM. Van Rijn§ and A.M.L. Coenen§

**Laboratori de Fisiologia, Universitat de les Illes Balears, Palma de Mallorca, Spain, †Department of Basic Physics, Universidad de La Laguna, Tenerife, Spain, ‡Laboratorio de Biofísica, Universidad de la Laguna, Tenerife, Spain and §Department of Biological Psychology/NICI, Katholieke Universiteit Nijmegen, The Netherlands*

Non-linear methods of time series analysis provide information on the physiological processes generating the EEG and can be used to recognize the interhemispheric EEG relationships in different vigilance states. In anaesthesia, there is low frequency EEG synchronization with an organized and distinguishable pattern which depends on the anaesthetic agent and the level of anaesthesia. The present work aims at analysing non-linear interdependencies between two symmetric EEG channels during the anaesthesia induction with propofol. The results will be contrasted with those obtained after univariate spectral analysis calculating the EEG power spectral densities within different frequency bands.

Fourteen patients aged 40 ± 15 years undergoing reconstructive surgery participated in this study, performed according to the guidelines of the local ethics committee for human studies. The anaesthesia was induced and maintained with propofol, given via a Target Controlled Infusion algorithm in a concentration range of 0.00–6.0 μg ml^{-1}. EEG was recorded with Ag–AgCl electrodes from C3–C4 according to the international 10–20 system. The interdependence between two reconstructed state spaces was made according to Arnhold *et al.* (1999) and Pereda *et al.* (2002). The real existence of interdependence was demonstrated by comparing the original value of the Arnhold's similarity index (S) with that obtained from 19 univariate surrogate versions of the time series. If the S value for the original X–Y pair results is greater than the value obtained for the 19 pairs of the original X-surrogate Y, then the difference is considered to be significant for $P < 0.05$ (Student's paired t test).

The S indexes showed a significant decrease ($P < 0.01$, ANOVA) in the interdependence between both electrodes when the dose surpassed 2.20 μg ml^{-1}. At this point, a marked inflexion occurred, showing that the dynamic behaviour of the EEG changed. Instead, applying the univariate spectral techniques the differences only appeared in the delta band at doses higher than 2.20 μg ml^{-1} ($P < 0.01$, ANOVA).

Our results agree with Zhang *et al.* (2001), who suggested that non-linear indexes show greater sensitivity than linear ones, especially when related to anaesthetic studies. A change in the dynamics of the EEG has been shown over a certain dose, which confirms the usefulness of the multivariate non-linear indexes to assess the depth of surgical anaesthesia.

Arnhold J *et al.* (1999). *Physica D* **134**, 419–432.

Pereda E *et al.* (2002). *IEEE Trans Biomed Eng* **49**, 548–555.

Zhang X *et al.* (2001). *IEEE Trans Biomed Eng* **48**, 1424–1433.

This work was partially supported by the FIS 020194, MCyT BFI2002–01159 and NGN-4372 of the NWO-STW.

All procedures accord with current local guidelines and the Declaration of Helsinki.