

Poster Presentations

Emotion and Stress

ES1

EFFECTS OF POSTNATAL HANDLING OF RATS ON ACUTE STRESS-INDUCED *c-fos* EXPRESSION IN LOCUS COERULEUS AND HYPOTHALAMIC PARAVENTRICULAR NUCLEUS

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Numerous studies have shown that postnatal handling (PH) of rat pups permanently alters hypothalamic-pituitary-adrenal (HPA) responses to stress. As adults, handled rats exhibit reduced secretion of ACTH and corticosterone in response to stress. These effects occur via an increase in glucocorticoid receptor density in forebrain neurons that control HPA activity which permits enhanced negative-feedback control of CRH synthesis and reduced ACTH release. Indeed, postnatally handled animals show decreased CRH levels compared with non-handled animals. The neurons located in the parvocellular region of the hypothalamic paraventricular nucleus (PVN) synthesize and release CRH. However, the consequences of early handling on other components of the central stress system are not known. The substantial involvement of the locus coeruleus (LC) in stress responses has been well documented in recent years. The LC, which is composed almost exclusively of noradrenergic neurons, interacts with other areas of the CNS (prefrontal cortex, amygdala, hippocampus, hypothalamus, mesolimbic and mesocortical dopaminergic system) that play an important role in information processing, action initiation, as well as setting the emotional tone. We are interested to know the effects of PH on this system involved in the generalized stress response. In the present experiment we have investigated whether PH could affect LC and PVN cellular activity during restraint stress. Expression of the immediate early gene *c-fos* was used as a marker of neuronal activation. Using Wistar rats, handling was performed once a day between 1 and 21 postnatal days. Nonhandled litters were left undisturbed until weaning. As adults, handled (H) and nonhandled (NH) male rats (400-500 g) were exposed to restraint stress for 2 hrs. Animals were sacrificed 60 min. after the stressful stimulus and their brains processed for immunocytochemical detection of Fos protein. As expected, H rats had a less number of Fos containing neurons in the parvocellular region of the PVN than did NH rats in response to restraint stress. However, an increased number of neurons expressing Fos-immunoreactivity in their nuclei was observed in LC of H rats compared with NH animals. These results confirm that a lower level of cellular activity in the PVN is a critical feature of the handling effect on HPA responses to stress and they suggest that the activity of LC noradrenergic neurons during stress is as well affected by this environmental manipulation. These findings support the importance of early experiences for the development of individual differences in neural systems involved in the stress response.

Supported by a grant from DGES (PB95-0050), Spain

ES2**EFFECTS OF EARLY HANDLING ON RAT BRAIN MONOAMINES AND ON THE ABILITY TO COPE WITH STRESS***A. Papaioannou¹*, F. Alikaridis¹, E. Bozas¹, K. Gerozissis², and F. Stylianopoulou¹**¹Laboratory of Biology-Biochemistry, University of Athens, Athens, Greece and ²ESA 7059 CNRS, U. Paris 7, 75251 Paris, France*

Early experiences affect brain development, with consequent effects on adult behaviour. Neonatal handling is an experimental paradigm for early experiences known to affect the programming of the hypothalamic-pituitary-adrenal axis, and as a result the ability of the organism to respond to novel, stressful stimuli. We exposed rats to neonatal handling from birth to day 22. Even 1 day of handling resulted in increased c-fos immunoreactivity in the hippocampus, which could reflect the cellular changes leading to the long-term consequences of early handling. When adult, a cohort of handled and non-handled animals were subjected to 5 min forced swimming either once (short-term stress) or daily for 15 consecutive days (long-term stress) and their total immobility time was recorded. Animals were then sacrificed and levels of plasma corticosterone and brain monoamines were determined by RIA and electrochemical detection following HPLC, respectively. Another cohort of animals was employed to evaluate the sensitivity of postsynaptic 5-HT_{1A} receptors, by measuring the hypothermic response to hydroxydipropylaminotetralin hydrobromide (8-OH-DPAT HBr), a 5-HT_{1A} receptor agonist. Early handling resulted in increased basal 5-HT levels in both the females and the males. Furthermore early handling increased postsynaptic 5-HT_{1A} receptor sensitivity in the males, while in the females it reduced it. Upon exposure to a 5 min forced swimming stress once, dopaminergic and serotonergic neurotransmission was decreased in handled animals, the decrease in the latter being larger in the females than the males. In both sexes no difference was found between handled and non-handled animals in corticosterone levels after the short-term stress. In contrast, in the long-term paradigm, male handled animals had shorter immobility times and higher corticosterone levels than non-handled, while the opposite held true among females: handling resulted in longer immobility times and lower corticosterone levels. Our results show that upon chronic exposure to stress, handled females failed to cope and resorted to "learned helplessness", a form of depression, in contrast to males, in which early handling increased their adaptability.

ES3**STRESS-INDUCED INHIBITION OF MESOACCUMBENS DOPAMINE RELEASE MEDIATES BEHAVIOURAL 'DESPAIR'**

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A number of recent evidences indicates that the mesoaccumbens dopamine (DA) system responds with biphasic changes of neurotransmitter release to stressors. An initial increase of DA release from accumbal terminals is followed by a progressive decrease below basal levels that lasts as far as the stressful condition is maintained. Enhanced mesoaccumbens DA release in stressful conditions is hypothetically related to defensive responses toward the aversive stimulus, whilst inhibition of DA release might be related to 'coping failure' and subsequent cessation of defensive attempts against the unavoidable/uncontrollable aversive stimulus (1). To test this hypothesis, we evaluated behavioural and mesoaccumbens DA responses to stress in two inbred strains of mice. Mice of the DBA/2 strain exposed to the forced swimming test (FST), presented a progressive increase of immobility and the classical biphasic stress response in terms of mesoaccumbens DA release (increase followed by decrease). Instead, C57BL/6 mice showed both an immediate immobility response and immediate inhibitory mesoaccumbens DA response to the test. Similar difference in the mesoaccumbens stress response between the two strains were also induced by restraint, indicating that activation of mesoaccumbens DA release in mice of the DBA/2 strains was not dependent on motor activation. On the other hand, amphetamine challenge (2.5 mg/kg) was by far more effective to enhance mesoaccumbens DA release in C57BL/6 mice than in DBA/2 mice. The latter effect replicated results previously obtained by *in vivo* intracerebral microdialysis, demonstrating that the opposite strain-dependent responses to stress were not due to the analytical method chosen for these experiments (*ex vivo*) or to a generic hyposensitivity of the mesoaccumbens DA system in C57BL/6 mice. Moreover, chronic treatment with a commontype tricyclic antidepressant prevented both the immobility and the inhibition of mesoaccumbens DA promoted by FST by C57BL/6 mice, indicating a strict relationship between the behavioural and the central response. Finally, stress-induced enhancement of mesocortical DA metabolism was anticipated in mice of the C57BL/6 strain in comparison with DBA/2 and reduced by the antidepressant treatment.

(1) Cabib & Puglisi-Allegra (1996) *Psychopharmacology*, 128:331.

ES4**ALTERED EXPRESSION OF THE CELL ADHESION MOLECULES NCAM, PSA-NCAM, AND L1, AFTER CHRONIC EXPOSURE TO RESTRAINT STRESS.**

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Previous studies showed that chronic stress produces hippocampal atrophy which might be associated to learning deficits in hippocampus-dependent tasks. Cell adhesion molecules (CAMs) are known to play a major role in neural plasticity, including learning and memory processes. Recently, the breakdown in the organization of key CAMs has been suggested as a possible mechanism underlying the generation of brain pathology. In this study, we evaluated whether different CAM molecules (NCAM, PSA-NCAM, and L1) might show altered expression in different brain areas as a consequence to chronic stress exposure. Male Wistar rats (250 g at the beginning of the experiments) were either stressed or left undisturbed for 21 consecutive days. Stress consisted on placing the animals in wire mesh restrainers for 6 h/day. After 1 day left undisturbed, half of the animals in each condition, were trained on the contextual fear conditioning task (a 5.5 min session in which three -1mA- electric shocks were delivered). Twenty-four hours later (day 24), animals were tested for conditioning and immediately sacrificed. Different brain areas (hippocampus, frontal cortex, striatum, amygdala, and hypothalamus) were dissected for the subsequent evaluation of CAMs using ELISA and Western blotting techniques. The results showed a major regulation of CAMs at the level of the hippocampus: stressed rats displayed reduced NCAM levels and increased PSA-NCAM and L1 levels. Chronic stress reduced NCAM expression at the striatum and PSA-NCAM expression at the frontal cortex, both effects being reversed by training rats in the contextual fear conditioning paradigm. These data shows CAM regulation, at the level of the hippocampus, as a molecular correlate of chronic-stress induced cognitive alterations. In addition, they suggest that other brain areas showing CAM modulation after chronic stress might also contribute to the behavioural alterations observed after the stress procedure.

Supported by a grant from the DGES (PM96-0015), Spain

ES5**CHRONIC STRESS INDUCES DIFFERENTIAL EFFECTS IN TWO HIPPOCAMPUS-DEPENDENT TASKS**

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Previous studies have shown that chronic stress results in hippocampal atrophy. There is a certain controversy in the literature regarding on whether these structural effects can result in cognitive

impairments in hippocampus-dependent tasks. In this study, we tested whether 21 days of chronic restraint stress (6 h/day in wire mesh restrainers) might influence subsequent learning on two hippocampus-dependent tasks, spatial orientation in the Morris water maze (Exp. 1) and contextual fear conditioning (Exp. 2). Male Wistar rats (250 g at the beginning of the experiments) were either stressed or left undisturbed for 21 consecutive days. In experiment 1, we evaluated the ability of rats to learn to locate a submerged platform in the water maze (2 m diameter). On days 23-26, they were given 3 training trials/day (120 sec; 30 sec intertrial interval) followed, on day 26, by a free-swim transfer test. Two days afterwards, they were submitted to two reversal learning sessions (3 trials/day; 1st session on days 29-30; 2nd session on day 31) in which the platform was located in a different pool quadrant. The results showed no differences between controls and stressed rats on spatial orientation learning. However, consistent deficits were found in stressed rats in the reversal learning sessions. They showed longer latencies to find the new platform location than controls, as well as clear signs of behavioural perseveration. In experiment 2, a new set of rats was tested for their ability to acquire contextual fear conditioning. Rats were tested on the conditioning chamber on days 23-24. Conditioning was established on day 23 by locating rats in a new chamber and delivering 3 electric shocks (1 mA, 60 sec interval). Testing was carried out on the following day, in which rats were exposed to the cage for 5 min. The results showed that chronic stress induced an increase in contextual fear conditioning, as indicated by higher freezing levels displayed by stressed rats during the retrieval test. In summary, these data question the direct relationship between stress and hippocampus-dependent learning impairments that has frequently been attributed to chronic stress. Whereas we did not find any deficits in spatial orientation learning after chronic stress, more subtle deficits in this task were revealed when evaluating reversal learning abilities. On the other hand, the effect of this stress procedure on contextual fear conditioning, instead of being deleterious, was facilitating, which suggests that the cognitive effects induced by chronic stress on hippocampus-dependent tasks might be dependent on a number of factors associated with the task (i.e., motivational and/or emotional factors, behavioural requirements, etc.).

Supported by a grant from the DGES (PM96-0015), Spain.

ES6

BEHAVIOURS TEMPORALLY RELATED WITH ULTRASONIC VOCALISATIONS IN THE INFANT MOUSE (*Mus musculus*)

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The most used approach to investigate emotional responses in mice during early ontogeny is the analysis of the ultrasonic vocalisations (UVZs). The characterisation of pup behaviour during calling could provide useful information to perform a more accurate analysis of this phenomenon. Moreover, this approach could improve the power of ultrasonic vocalisation analysis in revealing subtle and limited

changes during emotional development. To this purpose, we performed an analysis of the temporal sequence of behaviours shown by mouse pups (postnatal day 7, the peak-emission day for CD-1 mouse strain) while isolated from both dam and litter to investigate the sequences with which behavioural items and ultrasound emissions occur. The four behavioural items that occurred more often were analysed: locomotion, head rising, wall climbing, and lying still. The variables considered were: probability of emitting, latency to emit, probability of interrupting, and duration of UVZ. The results showed a marked emission of UVZs in conjunction with behavioural items involving movement, and evidenced that the different variables analysed are differently modulated within each behavioural item. These findings provide a useful key to better understand the ultrasonic vocalisation *per se* and as a measure of emotional development in the mouse. Furthermore, UVZ production will be discussed in terms of the ecology of mother/offspring relationship in rodents, since the data support the hypothesis of the communicative role of these vocalisations.

ES7

A COMPREHENSIVE STUDY IN THE RAT OF THE SUITABILITY OF A SINGLE 24-HOUR MATERNAL SEPARATION EXPERIENCE AS A NEURODEVELOPMENTAL MODEL OF SENSORIMOTOR GATING AND SELECTIVE ATTENTION DEFICITS

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Schizophrenia patients often demonstrate deficits in prepulse inhibition (PPI) and latent inhibition (LI), indicating compromised sensorimotor gating and selective attention, respectively. Recently, rat models of deficits in PPI and LI have attempted to depart from pharmacological manipulations and these studies have been influenced by the human evidence that disturbances in normal brain development may induce schizophrenia, leading to the neurodevelopmental hypothesis of schizophrenia. In accordance with this hypothesis, models based on postnatal environmental manipulations have been proposed, including litter-dam separation ("maternal separation", MS) performed for a single 24-hr period, followed by PPI and LI assessment in adulthood. Against this background, we have conducted a comprehensive study of the effects of MS on PPI and LI in adult Wistar rats. Based on the evidence that infant rats demonstrate a stress hyporesponsive period (SHRP, days 3-14) and that the neurodevelopmental consequences of postnatal environmental manipulations are age-related, MS was performed at three different stages of development. That is, MS was conducted with male and female subjects aged 4 (MS4), 9 (MS9) or 18 (MS18) days, which were then tested, together with non-manipulated controls as adults. MS effects were studied on PPI of the acoustic startle response, and on three different LI paradigms, namely, conditioned taste aversion (CTA), active avoidance (AA) and conditioned emotional response (CER). MS did not affect startle amplitude or PPI of the acoustic startle response (ASR). Interestingly, there was an overall sex difference, with males demonstrating both enhanced ASR and PPI relative to females. In terms of LI,

MS did have an effect on LI, but this was dependent on the paradigm in which LI was studied and on the age of subjects at MS: In AA, MS18 disrupted LI in males, whereas in MS4 males a severe overall learning deficit precluded LI; in CER, there was no effect of MS on LI; in CTA, MS9 subjects actually demonstrated enhanced LI. Taken overall, this comprehensive study of the effects of MS on PPI and LI in the rat has provided strong evidence that a single 24-hr maternal separation in rat pups does not lead to reliable deficits in PPI or LI and, therefore, does not represent a robust environmental model of either the sensorimotor gating or selective attention deficits presented by sufferers of schizophrenia.

Supported by a grant from The Swiss Federal Institute of Technology Zurich.

ES8

HORMONAL AND BEHAVIOURAL HYPORESPONSIVITY TO BOTH FORCED NOVELTY AND D-AMPHETAMINE IN PERIADOLESCENT COMPARED TO ADULT MICE

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An individual variability in the function of the hypothalamic-pituitary-adrenal (HPA) axis has been involved in the sensitivity to amphetamine (AMPH) effects. Also, it has been recently suggested that psychostimulants experience may have a greater addictive potential among human adolescents than in adults. The hypothesis of age-related differences in the HPA response to both stress and psychostimulants has been here tested in an animal model of adolescence. Periadolescent (pnd 33-43) and Adult (pnd > 60) mice of both sexes were injected with AMPH (0, 2, or 10 mg/kg i.p.) and immediately faced with a mild stressful experience, such as a novel environment. Each animal was sacrificed at a different time-point (before the injection, NT group; 15, 30, or 120 min after the injection) and trunk blood collected. Basal corticosterone (CORT) levels in the NT group resulted consistently higher in periadolescents than in adults. The forced exposure to novelty was associated with a marked increment of both locomotion and plasma CORT levels in mice of both ages. However, age-related differences appeared, with SAL-injected periadolescent subjects showing a significantly lower HPA axis response to forced novelty - - in terms of CORT levels - - than adults, and being still hyperactive at the end of the 120-min session. Also, the AMPH 2 dosage produced both the expected hyperactivity profile and an increase in CORT secretion in adult animals, whereas it was much less effective in periadolescents. Upon an AMPH 10 administration, periadolescents exhibited a marked locomotor hyperactivity, but failed to show the stereotyped behavioural syndrome, that was typical of adults. The present results suggest an age-related peculiarity both in the function of HPA axis and in behavioural regulations during periadolescence. Animals of this age were characterized by elevated basal CORT levels and behavioural hyperactivity, whereas a hyporesponsivity profile appeared following both the stress of forced novelty and the AMPH challenge. Such age-related differences should be considered in the frame of the issue of psychobiological risk factors possibly involved in vulnerability to drug abuse during human adolescence.

ES9**ASSOCIATION OF PLASMA CHOLESTEROL LEVELS AND ACTIVE AVOIDANCE IN AGEING MICE: MODULATION BY HOUSING CONDITION**

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There is an increasing evidence that cholesterol levels can affect a variety of behaviours in both animals and humans. However, the direction of the relation between cholesterol levels and learning in animal models is controversial. There are studies showing an impairment of cognitive function after increasing-cholesterol treatments and, on the other hand, data showing an improvement of learning abilities as a consequence of cholesterol pellets implantation. Cholesterol effects on behaviour are supposed to be mediated by different mechanisms such as changes in membranes fluidity, serotonergic function and neuroactive steroids synthesis. Since social factors exert a strong influence on these potential mediators the aim of the present preliminary study was to investigate the role of isolation and social housing on the relation between plasma cholesterol levels and performances in shuttle-box avoidance acquisition in 1-year old male NMRI mice. Subjects were either isolated for 4 months or housed in groups of 4-5 animals. Active avoidance performances were measured in mice subjected to five daily 100-trial training sessions. Only animals reaching a criterion of 10% of avoidance responses in the last session were considered in the statistical analysis. A week later the animals were anaesthetised and blood samples were collected and tested for total cholesterol levels.

Results showed that mean cholesterol levels did not differ in the two experimental groups, while mice belonging to the social group performed better in the avoidance task in comparison to their isolated counterpart. Moreover, in isolated mice cholesterol levels (mg/dl) were positively correlated with the mean percentage of correct responses in the active avoidance task ($\rho=0,63$), conversely in socially housed mice this association was negative ($\rho=-0,59$). All together these results suggest that cholesterol is associated with active avoidance performance in mice, however the direction of such a correlation is opposite in socially housed and isolated animals. This contradictory result can be due to the negative effects of isolation on learning. It is therefore possible that cholesterol plays a crucial role in improving learning ability in low-performer animals, whereas in social animals that scored better in avoidance high level of cholesterol are associated with a worse performance. Other variables such as social rank and emotionality, may modulate this latter association.

ES10**THE FAMILIAR CONDITION ENHANCES THE ANXIOTIC RESPONSE IN DBA/2 BUT NOT IN ICR ALBINO MICE IN THE LIGHT/DARK TEST**

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The light-dark test, previously described by Crawley (1), is considered an ethological model of neophobia and exploration in which a conflict exists between the tendency of mice to explore a novel environment and the aversive properties of a brightly-lit open field. In this experimental paradigm crossings between a dark (safe) and a brightly-lit (aversive) compartment of a box is measured and this behaviour is selectively increased by benzodiazepines (2). Extensive literature describes the results obtained with different experimental conditions and different mouse strains in order to obtain a consistent baseline level of exploratory activity of the control groups (3,4). We tested two strains of mice namely ICR (CD-1) albino and DBA/2 (Harlan). The first strain is widely used in different experimental procedures and is characterized by a high level of anxiety, while DBA/2 strain shows a low response to anxiogenic stimuli and is widely used in behavioural pharmacology (5). Three different experimental conditions have been used: A) mice were non-manipulated, B) mice were tail suspended for 5 min before light/dark test in a dark condition to increase the anxiogenic component and C) mice were previously exposed (96h and 48h) to the non-lighted test box to reduce the anxiogenic effect of a new environment. We observed that the behavioural pattern of the non-manipulated groups differs among strains. In particular DBA/2 strain has a latency significantly higher than albino strain, thus showing a less anxious behaviour, while the exploratory activity (line crossings, rearings and transitions) is significantly lower than in albino mice. Moreover DBA/2 mice are more responsive to variations of experimental conditions than albino mice and the responses of this strain are more reproducible. The familiar condition induces a significant decrease of latency time in the DBA/2, respect to non-manipulated group, with a concomitant significant increase of number of rearings, line crossings and transitions. On the contrary, the tail suspended animals show a significant decrease of locomotor activity. These results indicate that the familiar condition enhances the exploratory behaviour, and that the aversive response to the anxiogenic element (the bright lit) is amplified by the familiarity with the test box.

(1) Crawley (1981) *Pharmacol. Biochem. Behav.*, 15:695; (2) Green. & Hodges (1991), *Behavioural Model in Psychopharmacology* P. Willner (ed); (3) Costall *et al.*(1989) *Pharmacol. Biochem. Behav.* 32:777; (4) Sánchez (1997) *Eur. Neuropsychopharmacol.*, 7:283; (5) Crawley (1997) *Psychopharmacol.*, 132:107.

ES11**THE ROLE OF THE HIPPOCAMPUS AND ENTORRHINAL CORTEX IN BEHAVIOURAL DISINHIBITION TO NOVELTY**

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Adaptive behavioural responses to repeated daily testing in the open field test were studied in naive rats. A clear-cut progressive habituation of exploratory activity was observed over the five daily sessions, but was more pronounced in HR (high responders to novelty, ambulatory scores above the median) than in LR rats (low responders, ambulatory scores below the median). Autoradiography revealed a significant greater specific binding of the GABA_A receptor agonist ³H-muscimol, to the entorhinal cortex and hippocampus (dentate gyrus and CA3 layer) of LR than to the same brain structures of HR rats. Selective depletion of brain serotonin (p-chlorophenylalanine pretreatment) abolished exploratory motor activity habituation in the open field test and decreased ³H-muscimol binding to the hippocampal and entorhinal cortex brain slices. It is concluded that the GABAergic innervation of the hippocampus and entorhinal cortex contributes to the habituation of rats exploratory activity probably by decreasing the aversive properties of novel stimuli. Moreover, brain serotonin seems to play a permissive role in the GABAergic control of this phenomenon.

ES12**DOES CLOMIPRAMINE (TRICYCLIC ANTIDEPRESSANT) REALLY INDUCE A DECREASE OF MOTOR ACTIVITY IN RATS?**

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Generally, previous reports showed that acute or chronic injections of CMI and other TCAs first decrease the motor activity of rodents (Jahkel M. et al. 1994), second mitigate and even counteract the consequences of acute or chronic stress (Martin P. et al. 1990 ; Zehrowska-Lupina et al. 1992). Other results are not in agreement (Noda Y. 1997) and even opposite (Rodriguez E.L. 1982). In taking into consideration factors liable to be responsible of discrepancies (moment of experiment in circadian rhythm, dose of TCA daily injected, fine definition of behavioural units of exploration, genetic differences), the aim of this work was, on the one hand, to evaluate the effects of subchronic injections of CMI on locomotor and exploratory behaviours, on the other hand to verify the ability of CMI to counteract the behavioural effects of stress induce by a non physical aggressive situation. Motor activity of 75 female rats was registered on the classical open-field situation and their exploratory behaviour by means of a new exploration situation using hanged up objects to catch the eye of the animal (Decaris E. et al. 1997). CMI was injected twice a day during 7 days (20 mg/kg/day). After a period of training to

avoid bright light by pressing a lever, stress was obtained from a random reward. Our results showed first that subchronic but non acute injections of CMI reduced the exploratory behaviour of rats without modifying significantly their global motor activity. This decrease should not be due to an anxiolytic effect of the drug. Second, stress and CMI both decreased the exploration and their effects were paradoxically additive : then CMI injections did not counteract the stress consequences. From our results, from other experimental data (Martrette J.M. 1999) and from clinical observations in depressed humans treated with antidepressant the hypothesis that TCAs, like CMI, act specifically on attentional and /or motivational processes is proposed.

ES13

INFLUENCE OF SODIUM VALPROATE ON ANXIOTIC RESPONSES IN MICE

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Available evidence derived from preclinical (1) and clinical (2) studies suggests that the anticonvulsant sodium valproate (VPA), a GABA agonist, can effectively treat anxiety disorders. Since the previous demonstrations of anxiolytic-like properties of VPA have used paradigms involving electric shock, hyponeophagia, elevated plus-maze and light/dark aversion test, the present experiments studies the anxiolytic propertie-like effects of VPA in the marble burying test in mice (3). This test measures burying by mice when glass marbles were used as the provoking stimulus. It has been suggested that burying behaviour could constitute a useful test for anxiolytic activity, since it was differentially inhibited by a variety of anxiolytic agents, including benzodiazepines (BDZs) at doses which do not reduce swim-induced grooming. Non-anxiolytic drugs reduced marble burying at doses which reduce swim-induced grooming in mice. In the present experiments, the chronic effects of VPA were compared with those of a BDZ compound. Pentylentetrazole (PTZ), at a subconvulsant dose, was selected as a prototype of anxiogenic drugs. Furthermore, drug effects on marble burying were compared with effects on locomotor activity and rearing in an open-field test in mice. Results showed that multiple doses of 300-400 mg/kg i.p. of VPA were able to reduce the anxiogenic-like behaviour of PTZ. In summary, this study provides further evidence for the role of VPA in the control of anxiety.

¹de Angelis, L (1992) *Drug Dev Res*, 25:331; ²Keck PE et al. (1993) *Biol. Psych.*, 33:542; ³Njung K & Handley SL (1991) *Pharmacol. Biochem. Behav.*, 38:63.

ES14**SHORT AND LONG-TERM BEHAVIOURAL EFFECTS OF MDMA ("ECSTASY") ON ADOLESCENT AND ADULT MICE OF BOTH SEXES***G. Laviola* and S. Morley-Fletcher**Section of Behavioural Pathophysiology, Lab. F.O.S., Istituto Superiore di Sanità, Roma, Italy*

During adolescence, the brain and hormonal systems are still undergoing crucial maturational rearrangements, which take place together with significant modifications in psychosocial development. Despite these considerations, the neurochemical, hormonal, and behavioural facets of adolescence have been poorly investigated in relation to the increased vulnerability to e.g., psychostimulants such as MDMA (3,4-methylenedioxymethamphetamine), a synthetic analogue of amphetamine and mescaline that has emerged as a popular recreational drug of abuse. A crucial issue here is also novelty-seeking, a temperamental/behavioural trait that is typical of adolescence and which might contribute to vulnerability from both a psychological and a psychobiological standpoint. In animal models, the search for novel stimuli actually shares a common neurobiological substrate with psychostimulant effects, i.e. the reward-related brain mesolimbic pathways. The aim of this study was to examine the acute and long-term effects of MDMA administration (0, 5 or 10 mg/kg treatment history) on different behavioural responses. Separate groups of male and female mice were assigned for treatment to three different developmental ages (early adolescence: pnd 28, late adolescence: pnd 38 or young adulthood: pnd 52), to determine if there are any age and gender-dependent differences in susceptibility to MDMA-induced changes.

Animals received a repeated and intermittent i.p. drug injection for three days. On the third day following drug administration, mice were assessed for pain sensitivity in a hot plate test ($55 \pm 1^\circ\text{C}$). A dose-dependent analgesia was found, which was more marked in late adolescents than in the other two age groups. When mice reached at least pnd 80, carry-over effects of juvenile drug experience were investigated for all subjects in a free-choice novelty paradigm. Animals underwent a familiarisation schedule for three consecutive Training days with one side of a two-chamber apparatus. On Test day 4, mice were challenged either with saline or a standard 5 mg/kg MDMA dose. Following the opening of a partition, which allowed mice to freely move from the familiar compartment to a novel one, all animals showed an increased arousal in their locomotor/exploratory activity as well as a clear-cut preference for the novel environment (Novelty Seeking). MDMA produced hyperactivity, and a dose-dependent sensitization was found for this parameter with a prominent increment of locomotion in the 10 mg/kg history group. The profile of Novelty Seeking was significantly more marked in subjects chronically injected with drug during early adolescence when compared to subjects treated when already young adults. This profile also resulted to be much evident in females. In conclusion, present results indicate that repeated MDMA experience during early adolescence has marked carry-over effects on both the response to novelty, where there are also slight gender differences, and to challenge with the same drug in adulthood. A better understanding of psychostimulant effects during adolescence on the complicated interaction between genetic, neurobiologic, psychosocial, and environmental factors will lead to earlier and more effective prevention and treatment.

ES15**THE DISTRIBUTION OF CRF IMMUNOREACTIVITY IN THE AVIAN BRAIN**

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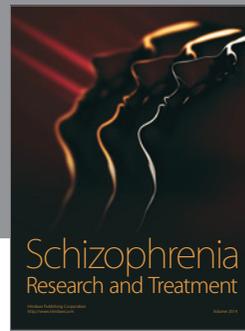
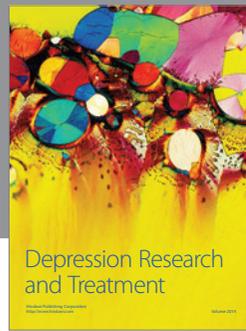
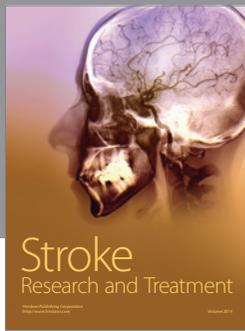
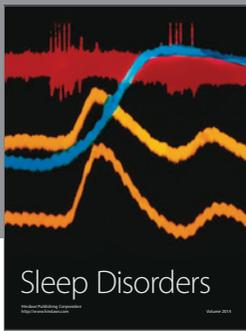
In birds as in mammals, the cerebral distribution of corticotropin-releasing factor (CRF) is not restricted to the hypothalamo-pituitary axis and CRF may play a role in fear/stress responses other than the control of adrenocorticotropin hormone release by the pituitary. The results of a number of studies suggest that CRF could be involved in the behavioural responses to a variety of stressors. However, the way in which CRF mediates these responses is not clear. Therefore, in order to provide a firm basis for the investigation of the role of CRF in the control of avian behaviour, the distribution of CRF-containing neurones and fibres throughout the brain was determined. Three domestic chicks (*Gallus domesticus*) and three adult Japanese quails (*Coturnix japonica*) were given a lethal dose of anaesthetic and perfused through the left cardiac ventricle with a flush of phosphate buffer (0.1M, pH 7.4), followed by 4% paraformaldehyde in the same buffer. The brains were removed from the skulls and immersed in fixative for 3-4 h, followed by immersion in a solution of 30% sucrose in phosphate buffer at 4°C until they sank. Serial, coronal sections (50µm) were then cut in a cryostat and processed for immunocytochemistry with a primary antibody raised against mammalian CRF (Peninsula Laboratories), using the avidin-biotin complex method. The resulting peroxidase labelling was developed using diaminobenzidine as a chromogen. Some sections were stained with cresyl violet to aid localisation of immunolabelling. The distribution of CRF-immunoreactive (ir) perikarya and fibres in the chick/quail brain was found to be far more extensive than previously reported, notably in the telencephalon. In particular, numerous CRF-ir neurones and fibres were present in the hyperstriatum (with a higher density in the hyperstriatum accessorium than in the hyperstriatum intercalatum supremum, hyperstriatum dorsale and hyperstriatum ventrale), hippocampus, neostriatum, lobus parolfactorius (mainly medially), archistriatum, nucleus taeniae and nucleus accumbens, which exhibited the strongest immunolabelling in the telencephalon. The septal region contained numerous CRF-ir fibres. The presence of dense populations of CRF-ir neurones and fibres in the archistriatum, nucleus accumbens and lobus parolfactorius strengthens the hypothesis of an "extended avian amygdala" including these three structures. Moreover, the presence of CRF in such structures considered to be "limbic" in nature, supports the hypothesis of involvement of extra-hypothalamic CRF in emotional responses.

ES16**A FEAR-ACQUISITION SYSTEM IN AMPHIBIANS***S.O.G. Lindemann* and G. Roth**Brain Research Institut, University of Bremen, 28334 Bremen, Germany*

Amphibians possess most limbic centers found in mammals including the hippocampal formation (medial pallium), amygdala, septum, ventral striatum, hypothalamus, nucleus accumbens, ventral tegmental area and reticular nuclei such as the nuclei raphes and the locus coeruleus (Northcutt, 1981; Marin et al, 1996). We found evidence that - as in mammals - in amphibians limbic centers play an important role in the acquisition of fear. To investigate the function of the limbic system regarding the acquisition of fear, we developed a one-trial aversive learning task in the fire-bellied toad, *Bombina orientalis* using honey bees (*Apis m.sicula*) as negative conditioning stimulus. For identification of the brain areas involved in this task, the distribution of Egr-1, a transcription factor encoded by the immediate early gene (IEG) *egr-1*, was investigated using immunohistochemistry. Egr-1 (also known as *krox 24*, *zif/268*, *ZENK*) as well as *c-fos* are supposed to play an important role in the consolidation of memory traces (Grimm et al., 1996; Jin et al., 1997). We found that the expression of Egr-1 in the medial amygdala, the bed nucleus of the stria terminalis and the preoptic area of the experimental group was significantly higher compared to controls fed with palatable food.

Posture and Movement**PM1****VESTIBULAR CONTRIBUTION TO UPDATING THE RETINOTOPIC MAP***J. Ventre-Dominey¹* and I. Israël²**¹INSERM, Vision et Motricité, Bron - ²CNRS, LPPA, Collège de France, Paris, France*

Our purpose was to investigate in human the updating mechanism of retinotopic map based on vestibular information. The novelty of our study was to compare the ocular position accuracy to a memorized visual target 1) when the vestibular input was taken into account to update the retinal error or 2) when the vestibular input was ignored in order to maintain constant the retinal error. We studied horizontal saccades to visual memorized targets in 7 healthy subjects. The subject was seated in a rotating chair in the dark and horizontal and vertical eye movements were recorded by DC current electro-oculography. The paradigm was composed of 3 different conditions as follows: First, the subject had to maintain the gaze onto a head-fixed visual fixation point (for 7.5 sec) while a visual target was flashed for 1 sec at 10 degrees on the horizontal axis. After the extinction of the fixation point the subject had to perform ocular saccades onto the memorized visual target location 1) either in the current stationary body condition (Visual Condition: ViC) or 2) after a chair velocity step rotation with head-fixed visual point fixation. In



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