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ENVIRONNEMENT PRENATAL ET CAPACITES ADAPTATIVES CHEZ LE RONGEUR : TROUBLES EMOTIONNELS ET VULNERABILITE AUX DROGUES

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LISTE DES PUBLICATIONS

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Morley-Fletcher S., Palanza P., Parolaro D., Vigano D., Laviola G. *Intrauterine position has long-term effects on mu opioid induced behaviours in mice.* Psychoneuroendocrinology (sous presse).

Morley-Fletcher S., Darnaudery M., Koehl M., Munoz C., Casolini P., Van Reeth O., Maccari S. *High corticosterone levels in prenatally stressed rats predict immobility behaviour in the forced swim test. Effects of a chronic treatment with tianeptine.* Brain Research (soumis).

Morley-Fletcher S., Rea M., Maccari S., Laviola G. *Environmental enrichment during adolescence reverses the effects of prenatal stress on anxiety-related behaviours and stress reactivity in rats.* European Journal of Neurosciences (soumis).

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Morley-Fletcher S., Darnaudéry M., Mocaer E., Froger N., Lanfumey L., Laviola G., Casolini P., Zuena A., Hamon M., Maccari S. *Chronic treatment with imipramine affects behavior, hippocampal corticosteroids and cortical 5-HT1A receptor expression in prenatally stressed rats* (en préparation).

SOMMAIRE

SUMMARY

The neurobiological and behavioural development of the individual is under the influence of both genetic and epigenetic factors. Early ontogeny is a markedly plastic and crucial stage, and alterations of prenatal milieu may have a great influence in modulating developmental trajectories that in turn will influence subsequent behavioural responses.

Aim of this PhD thesis was to evaluate in the rodent the impact of early experience on the development of maladaptive behaviours during adolescence and at adulthood. Different precocious experiences have been considered: a natural occurring phenomenon such as intrauterine position, which is known to affect the degree of exposure to sex hormones during the period of sexual differentiation; an induced phenomenon like exposure to stress in utero which is known to impair the hypothalamus pituitary adrenal (HPA) axis of the developing individual.

The impact of these two factors have been investigated adopting an integrated approach in the framework of an increased vulnerability to drugs of abuse during adolescence and to depressive-like disturbances in an animal model such as the prenatal stressed rat.

The intrauterine position (IUP) phenomenon represents a naturally occurring variation in degree of exposure to sex hormones during the prenatal phase of sexual differentiation as a consequence of the in-utero proximity to opposite sex fetuses. The IUP determines fetal hormone levels since endogenous sex steroids are transported from one fetus to another, thus modulating the organising action of sex hormones. This "in-utero sibling effect" has been shown to account for significant degree of the individual variation observed on different behavioural traits not only related to reproduction. In addition to the well-characterised prenatal and neonatal critical periods during which the brain is organised by sex steroids, the onset of puberty also represents an important developmental phase. During this period, together with the development of reproductive functions, individuals acquire mature survival skills that allow them to reach independence from parental care. Based on these considerations, we have conducted two studies in mice, aiming to investigate the interplay between the long-term effects of IUP and activational effects of circulating sex hormones which occur during adolescence on the expression of a sexually dimorphic behaviour such as novelty seeking behaviour in mice of both sexes and from known IUP. Adolescent rodents show elevated basal levels of explorative activity as compared to their adult subjects and,

are also characterised by an enhanced sensitivity to psychostimulants. Therefore, we have also considered the hypothesis that influence of IUP on novelty-seeking behaviour would be consistent with its influence on animal response to psychostimulants. The results obtained have evidence that uterine location between two males enhanced novelty seeking behaviour in males during adolescence, whereas in adulthood it increased density of midbrain μ-opioid receptors as well as it enhanced sensitivity to the analgesic effects of opioid administration. Therefore prenatal exposure to different physiological titers of sex hormones is able to modulate the process of early organisation of those brain systems that will then underlie the expression of novelty-seeking behaviour during adolescence and more later vulnerability to psychostimulants.

The individual vulnerability to the reinforcing properties of drugs plays an important part in the subsequent development of addiction. In addition to sex and age differences, exposure to stress plays a major role in determining an enhanced responsivity to drugs. Prenatally stressed animals have been shown to be more vulnerable to psychostimulants at the adult stage. We decided to investigate this aspect early at the adolescent stage. We have conducted a study aiming to assess the influence of prenatal stress on vulnerability to "ecstasy" (3,4-methylenedioxymethamphetamine, MDMA) in adolescent female rats (pnd 30). Following oral administration of MDMA a time-course analysis of animals' performance in a simple motor coordination task, as well as measurement of drug levels in the blood was carried out. Significantly constantly higher values of circulating MDMA, as well as episodes of drug-induced alterations of motor coordination were evidenced in the PS group than in non stressed animals. These results have confirmed a general higher vulnerability to drugs of these animals indicating that an altered drug metabolism could play a role in determining this profile.

As a whole, the results obtained in this first part have shown that two different models of early life experience that imply subtle (IUP phenomenon) or strong (PS) modifications of the prenatal environment had a long-term effects on individual strategies of coping with environmental challenges.

In a second part of this thesis, we have investigated the PS rat as an animal model of psychopathology and addressed the possibility to reverse its alterations by using two

different approaches: pharmacological and environmental. Both paradigms have proven to be effective in reversing PS induced abnormalities at the behavioural, endocrinological and neurochemical level.

When adopting a pharmacological approach, we have considered the need to treat chronically the PS rats with antidepressant to parallel the clinical outcomes which indicate that effects of antidepressant drugs appear no earlier than two or three weeks after the onset of treatment.

In a first study, we have evaluated if the dysfunctions at the levels of the HPA axis that are characteristic of PS rat, could predict an impaired behaviour in the forced swim test, a test classically used to validate antidepressant activity. We have observed a positive correlation between corticosterone levels and behavioural performance in the forced swim test. PS rats that exhibited an HPA axis hyperactivity spent more time in passive behaviour with respect to controls. Following a chronic treatment with tianeptine, the immobility behaviour was markedly reduced.

In the second study, we extended the investigation of the predictive validity of the PS rats, focusing our attention on the effects of a chronic treatment with imipramine on anxiety behaviour, HPA axis and serotonergic system. PS rats were characterised by high levels of self-grooming behaviour when faced with a conspecific and by a reduced exploration of the open arms in the elevated plus-maze test, thus confirming a general profile of anxiety in these animals. Chronic treatment with imipramine had a slight anxiolytic effect in the social interaction test but no effect on the elevated plus-maze response.

When assessed in the forced swim test, PS rats were more immobile than controls. Once again, following treatment with imipramine, the immobility behaviour was markedly reduced. Normalisation of HPA axis and 5-HT system by imipramine was concomitant with increased levels of hippocampal corticosteroid and decreased levels of 5-HT1A mRNA, which is consistent with the efficacy of pharmacotherapic intervention on human depression.

When adopting an environmental approach we have used an enriched environment in which PS animals have been reared from weaning up to the end of the adolescent period. Animals were assessed during the adolescence phase since this is a highly plastic stage of development that can be more sensitive than others to environmental manipulations. The effects of the environment were assessed at the levels of play behaviour which is markedly expressed by animals around this age, emotionality in the elevated plus-maze and HPA axis

response to an acute stress.

PS adolescent rats showed reduced play behaviour, increased anxiety in the elevated plus maze and prolonged corticosterone secretion in response to restrain stress. Environmental enrichment increased play behaviour and reduced anxiety behaviour in the elevated plus Moreover, PS enriched rats showed a reduced peak and a return to baseline levels similar to controls, thus indicating an improved regulation of the HPA axis. Interestingly, environmental enrichment had no effect on corticosterone secretion in the control group. As a whole, these results indicate that rats exposed prenatally to stress can benefit from the modulatory effects of an enriched environment during adolescence.

As a whole, the results obtained in this second part have shown that the PS model in the rat could be a suitable animal model for the design and testing of new therapeutic strategies in mood disorders as a function of early insults.

The studies conducted in this thesis work evidence the importance of considering the impact of subtle and/or strong perturbations in the early environment of the developing individual, as programming factors that can regulate the expression of its future adaptive capabilities. Moreover, it indicates the need to adopt an integrated approach in the evaluation of therapeutic interventions.

RESUME

Le développement neurobiologique et comportemental d'un individu est influencé par des facteurs génétiques, mais aussi par des facteurs épigénétiques. L'ontogenèse précoce est fortement plastique et constitue un stade crucial du développement. Les altérations de l'environnement prénatal peuvent avoir une grande influence sur la modulation du développement qui, en retour, pourra influencer les futures réponses comportementales.

Le but de cette thèse était d'évaluer l'impact des expériences précoces sur le développement de comportement non adaptatifs à l'adolescence et à l'âge adulte, chez le rongeur. Diverses expériences précoces ont été considérées : un phénomène naturel tel que la position intra-utérine, qui affecte le degré d'exposition aux hormones sexuelles au moment de la différentiation sexuelle, et un phénomène induit tel que l'exposition au stress in utero, qui altère l'axe hypothalamo-hypophyso-surrénalien (HHS) de l'individu en développement.

L'impact de ces deux facteurs à été examinée en adoptant une approche intégrée étudiant tout d'abord l'augmentation de la vulnérabilité aux drogues à l'adolescence et ensuite, les troubles comparables à la dépression dans le modèle animal du rat stressé prénatalement.

Le phénomène de la position intra-utérine (PIU) représente une variation naturelle du degré d'exposition aux hormones sexuelles lors de la phase prénatale de la différentiation sexuelle. Ce phénomène résulte de la proximité in utero de fœtus de sexes opposés. La PIU détermine les taux d'hormones fœtales car les stéroïdes sexuelles endogènes sont transportées d'un fœtus à l'autre, modulant ainsi l'action organisatrice de ces hormones. Cet « effet de fratrie in utero" rend compte significativement de la variation individuelle observée sur différents traits comportementaux, ces traits n'étant pas exclusivement associés à la reproduction. Ajouté aux périodes prénatales et néonatales, phases critiques dans le développement, durant lesquelles le cerveau est organisé par les stéroïdes sexuelles, le début de la puberté constitue également une phase importante dans le développement. Lors de cette période, et en parallèle avec le développement des fonctions reproductrices, les individus acquièrent des habiletés indispensables à leur survie, qui leur permettent d'atteindre l'indépendance vis à vis de leurs parents. A la lumière de ces considérations, nous avons conduits deux études chez la souris. Ces études avaient pour but d'examiner l'interaction entre les effets à long terme de la PIU et les effets activateurs des hormones sexuelles circulantes. Ces hormones agissent lors de l'adolescence sur l'expression du comportement sexuellement dimorphique tel que le comportement de recherche de nouveauté chez la souris, et ce, quelque soit le sexe et à PIU connue. Les rongeurs

adolescents montrent des niveaux élevés d'activité exploratoire comparés aux adultes et sont également caractérisés par une sensibilité accrue aux psychostimulants. Nous avons donc considéré l'hypothèse que l'influence de la PIU sur le comportement de recherche de nouveauté pourrait être liée à son influence sur la réponse des animaux aux psychostimulants. Les résultats obtenus montrent qu'une position utérine entre deux mâles augmente le comportement de recherche de nouveauté chez les mâles lors de l'adolescence, alors que à l'âge adulte, cela augmente la densité des récepteurs μ du mésencéphale et la sensibilté aux effets analgésiques de l'administration d'opiacées. L'exposition prénatale à différents taux physiologiques d'hormones sexuelles module les processus d'organisation précoce de ces systèmes cérébraux qui pourront alors soutenir l'expression du comportement de recherche de nouveauté lors de l'adolescence et plus tard, la vulnérabilité aux psychostimulants.

La vulnérabilité individuelle aux propriétés renforçatrices des drogues joue un rôle important dans le développement ultérieur de l'addiction. En plus des différences de sexe et d'âge, l'exposition au stress joue un rôle majeur dans l'augmentation de la sensibilité aux drogues. Il a été démontré que les rats stressés prénataux (SP) sont plus vulnérables aux psychostimulants à l'âge adulte. Nous avons donc décidé d'examiner cet aspect de façon précoce, à l'adolescence. Nous avons conduit une étude visant à évaluer l'influence du stress prénatal sur la vulnérabilité à l"ecstasy" (3,4-methylenedioxymethamphetamine, MDMA) chez des rats femelles adolescents (âgées de 30 jours). Après l'administration orale de MDMA nous avons conduit une analyse au temporelle des performances des animaux dans une tâche de coordination motrice simple et nous avons également mesuré les taux sanguins de drogue. Des valeurs significativement élevées de MDMA circulant, ainsi que des altérations de la coordination motrice induites par la drogue ont été objectivées dans le groupe SP. Ces modifications étaient absentes chezon chez les animaux non stressés. Ces résultats ont confirmé la vulnérabilité aux drogues plus élevée chez ces animaux, indiquant que le métabolisme altéré de la drogue pourrait jouer un rôle dans la détermination de ce profil.

De façon générale, les résultas obtenus dans cette première partie ont montré que les deux modèles d'expérience précoce : impliquant des modifications soit subtiles (le phénomène de la PIU) soit fortes (le SP) de l'environnement prénatal ont des effets à long terme sur les stratégies individuelles d'adaptation aux conditions environnementales (« *coping* »).

Dans la seconde partie de cette thèse, nous avons étudié le rat SP comme un modèle animal de psychopathologie et nous nous sommes questionnés sur la possibilité de

réversion de ces altérations par l'utilisation de deux approches : une approche pharmacologique et une approche environnementale. Les deux paradigmes se sont montrés efficaces dans la réversion des effets du SP aux niveaux neurocomportemental, endocrinien et neurochimique.

En ce qui concerne l'approche pharmacologique, nous avons considéré la nécessité de traiter de façon chronique les rats SP avec des antidépresseurs pour être au plus proche des données cliniques qui indiquent que les effets des antidépresseurs n'apparaissent pas avant deux ou trois semaines après le début du traitement.

Dans la première étude, nous avons évalué si les dysfonctions au niveau de l'axe HHS, caractéristiques des rats SP, pourraient prédire un comportement altéré dans le test de la nage forcée, un test classiquement utilisé pour valider les propriétés antidépressives d'une molécule. Nous avons observé une corrélation positive entre les taux de corticostérone et la performance comportementale dans le test de la nage forcée. Les rats SP qui présentaient une hyperactivité de l'axe HHS, passaient plus de temps en comportement passif par rapport aux contrôles. Après un traitement chronique à la tianeptine, le comportement d'immobilité était fortement diminué.

Dans la seconde partie, nous avons étendu notre investigation à la validité prédictive du modèle de SP, en focalisant notre attention sur les effets d'un traitement chronique à l'imipramine sur le comportement anxieux, l'axe HHS et le système sérotoninergique. Les rats SP ont été caractérisés par des niveaux élevés de comportement de toilettage ("self-grooming") lorsqu'ils sont mis en présence d'un congénère et par une diminution de l'exploration des bras ouverts dans le test du labyrinthe en croix surélevé, confirmant ainsi le profil d'anxiété de ces animaux. Un traitement chronique à l'imipramine avait un faible effet sur le test d'interaction sociale mais n'avait pas d'effets sur la réponse au labyrinthe en croix surélevé. I

Lorsque les rats SP étaient examinés dans le test de la nage forcée, ils étaient plus immobiles que les contrôles. Une fois encore, après le traitement à l'imipramine, le comportement d'immobilité était très réduit. La normalisation de l'axe HHS et du système sérotoninergique fut concomitante à une augmentation des taux de corticostéroïdes hippocampiques et à une diminution des taux d'ARNm 5-HT1A, ce qui est en accord avec l'efficacité de la pharmacothérapie chez les patients dépressifs.

Lorsque nous avons adopté une approche environnementale, nous avons utilisé un environnement enrichi dans lequel les animaux ont été élevés du sevrage à l'adolescence. Les animaux ont été évalués lors de l'adolescence car cette période constitue un stade très plastique du développement qui peut être plus sensible que les autres aux manipulations environnementales. Les effets de l'environnement furent

évalués au niveau du comportement de jeu, très exprimé par les animaux à cet âge, le comportement émotionnel dans le labyrinthe en croix surélevé et la réponse de l'axe HHS à un stress aigu.

Les rats adolescents SP montraient une diminution du comportement de jeu, une augmentation de l'anxiété dans le labyrinthe en croix surélevé et une prolongation de la sécrétion de la corticostérone en réponse à un stress de contention. L'environnement enrichi a augmenté le comportement de jeu et a diminué le comportement d'anxiété dans le labyrinthe en croix surélevé. De plus, les rats SP élevés dans un environnement enrichi présentaient une diminution de la valeur du pic et un retour au niveau de base similaires à ceux des contrôles, indiquant une régulation altérée de l'axe HHS. De façon intéressante, l'environnement enrichi n'a pas d'effet sur la sécrétion de corticostérone dans le groupe contrôle. Ces résultats indiquent que les rats exposés au stress lors de la période prénatale peuvent bénéficier des effets modulateurs d'un environnement enrichi lors de l'adolescence.

De façon générale, les résultats de la seconde partie ont montré que le modèle du rat SP pourrait être un modèle approprié pour la conception et l'essai de nouvelles stratégies thérapeutiques dans le cadre des troubles de l'humeur résultant de facteurs précoces.

Les études conduites dans ce travail de thèse ont fourni des arguments en faveur de l'importance de l'impact des perturbations subtiles et/ou intenses dans l'environnement précoce d'un individu en développement, telles que les facteurs programmateurs qui peuvent réguler l'expression des futures capacités adaptatives. De plus, ces résultats soulignent la nécessité d'adopter une approche intégrée dans l'évaluation des interventions thérapeutiques.

INTRODUCTION

INTRODUCTION

I. LE PHENOMENE DE LA POSITION INTRA-UTERINE

Chez les espèces à gestation longue, telles que l'homme, la différenciation sexuelle est terminée à la naissance, alors que chez les espèces à gestation plus courte, telles que la souris et le rat, elle commence lors du dernier tiers de la gestation et se poursuit jusqu'à une semaine après la naissance (Gorski, 1979). Chez la souris, la différenciation sexuelle débute au treizième jour de gestation. La gestation dure 19 jours et un pic de secrétions des hormones gonadiques se produit au jour 17 (Rugh, 1968). La différenciation hypothalamique commence également lors de la dernière partie de la gestation, et se poursuit pendant la période néonatale. Chez le rat, ces événements se produisent environ deux jours plus tard que chez la souris et la gestation dure 22 jours (Weisz et Ward, 1980).

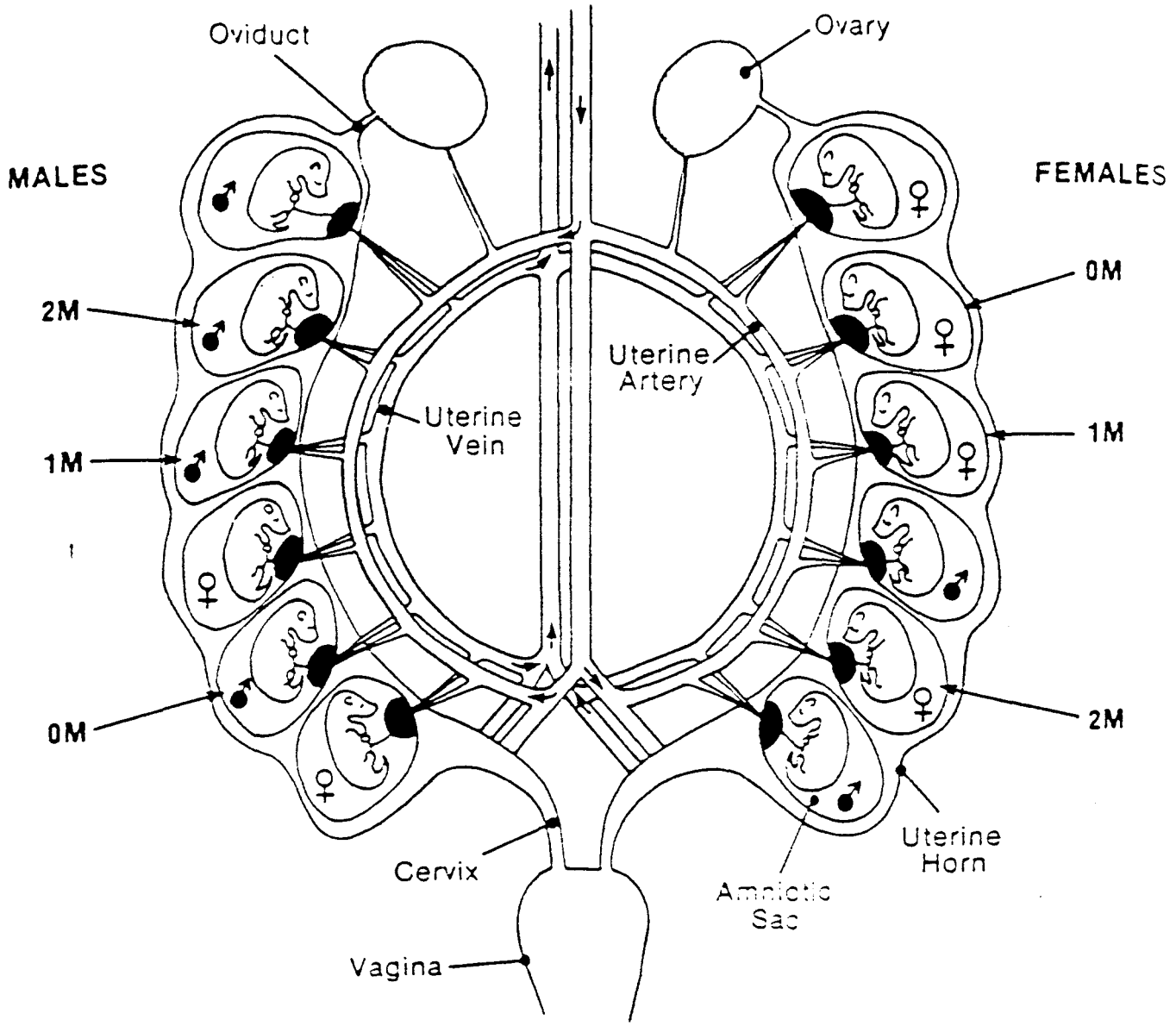
Bien que la secrétions de testostérone par les testicules fœtales soit nécessaire pour une masculinisation complète, l'œstradiol influe également la différenciation de certains caractères masculins (Gorski et Cannon, 1979 ; MacLusky et Naftolin, 1981). L'hypothèse de l'aromatisation propose que la testostérone secrétée par les testicules du fœtus ou du nouveau-né mâle agit dans le but de masculiniser (engendrant des caractères mâles) et de défiminiser (inhibant les caractères femelles) les régions cérébrales du cerveau en développement après avoir été convertie en œstrogène par l'aromatase (Mc Ewen et al., 1977).

Un corollaire de cette hypothèse est que les œstrogènes circulants, et notamment le 17ß-oestradiol, n'ont pas de rôle dans le processus de différenciation sexuelle (MacLusky, 1988). Ceci serait dû à la présence de concentrations très élevées de glycoprotéines plasmatiques telles que l'alpha-foetoprotéine qui se lie à l'œstradiol avec une très haute affinité chez le rat et la souris (Lai et al., 1976).

Les fortes concentrations plasmatiques d'alpha-foetoprotéine réduisent le passage de l'œstradiol dans les tissus pendant la différenciation sexuelle en diminuant la fraction libre d'œstradiol plasmatique, biologiquement active, à une concentration inférieure à celle capable d'exercer un rôle physiologique (Keel et Abney, 1984).

On pensait que, lors de la différenciation sexuelle, seul l'œstrogène résultant de l'aromatisation de la testostérone se liait à son récepteur et pouvait donc exercer son action au niveau cérébral. Cependant, à l'heure actuelle, plusieurs études ont montré que l'alpha-foetoprotéine liée à l'œstrogène peut passer la barrière hémato-encéphalique (pour revue, voir Fitch et Denenberg, 1998), et que l'œstrogène est directement impliqué dans l'organisation du cerveau et du comportement, ceci implique que la féminisation est un processus actif (Toran-Alleret, 1984 ; Fitch et Denenberg 1998 ; Gupta, 2000).

Intrauterine Position



The uterine horns and uterine loop arteries and veins of a pregnant mouse at term. Intrauterine position is determined at caesarean delivery. The labels 0M 1M and 2M refer to the number of male fetuses to which an individual is contiguous (2M=between 2 males, 1M= between a male and a female, 0M=between 2 females). The same classification scheme is used for both males and females. From vom Saal and colleagues (1990).

Montano et ses collaborateurs (1995) ont montré que, chez le rat, les taux sanguins d'œstradiol libre du fœtus et du nouveau-né femelles sont comparables aux taux physiologiquement actifs chez la femelle adulte et que, à ces valeurs, l'œstradiol circulant peut entrer dans les cellules du cerveau et se lier à son récepteur nucléaire. C'est pourquoi, l'hypothèse que l'"absence de deféminisation" chez la femelle résultait du fait que ces processus ne se produisent pas lors de la vie prénatale mais se mettent en place lors de la période post-natale précoce, lorsque les taux sanguins de testostérone sont beaucoup plus faibles, a été émise.

Chez les rongeurs, le phénomène de la position intra-utérine implique que la variation phénotypique chez des individus du même sexe pourrait dépendre du fait que, lors du développement intra-utérin, l'animal sera côte à côte avec un animal du même sexe ou du sexe opposé. La position intra-utérine (PIU) affecte la transmission des hormones sexuelles par un fœtus vers les fœtus qui lui sont côte à côte, modulant ainsi son exposition à la testostérone et à l'œstradiol lors de la période prénatale. La classification adoptée fait référence au nombre de fœtus mâle à coté duquel est un individu, cela est utilisé pour les deux sexes. Ainsi, un fœtus entre deux mâles sera un fœtus 2M, et un fœtus entre deux femelles sera un fœtus 0M (voir figure).

Chez la souris, Au jour 17 de gestation, le mâle a trois fois plus de testostérone circulant que la femelle, alors que la femelle plus d'œstradiol que le mâle. Un fœtus femelle 2M a plus de testostérone dans le sang et dans le liquide amniotique qu'un fœtus femelle 0M (vom Saal et Bronson, 1980a) et un fœtus mâle 0M possède plus d'œstradiol qu'un fœtus mâle 2M (vom Saal et al., 1983). De telles différences disparaissent lorsque les animaux sont adultes.

A. Influence de la position intra-utérine sur la physiologie

Chez la souris, au jour 17 de gestation, le mâle a trois fois plus de testostérone circulant que la femelle, alors que la femelle a plus d'œstradiol que le mâle. Un fœtus femelle 2M a plus de testostérone dans le sang et dans le liquide amniotique qu'un fœtus femelle 0M (vom Saal et Bronson, 1980a) et un fœtus mâle 0M possède plus d'œstradiol qu'un fœtus mâle 2M (vom Saal et al., 1983). Ces différences disparaissent lorsque les animaux sont adultes.

Vom Saal et Dhar (1992), ont montré que le flux sanguin dans l'artère utérine chez les rats était bidirectionnel, et que le transport des stéroïdes entre les fœtus d'une même portée se réalise selon le mode de diffusion dans la lumière utérine et non pas par les vaisseaux de l'utérus de la mère. Au jour 17 de la gestation, du carbone coloré a été injecté dans le cœur

d'une femelle gestante pour déterminer la direction du flux sanguin dans la veine utérine, alors que l'injection de colorant dans le placenta est utilisée pour déterminer la direction du flux sanguin dans la veine utérine. Il a été observé que le sang entre dans l'artère utérine au niveau des extrémités dorsale et caudale et que le flux sanguin veineux placentaire adopte une direction rostrale du côté de la portion rostrale de la corne utérine et une direction caudale du côté de la portion caudale de la corne utérine. Les auteurs ont conclu que le flux sanguin dans les vaisseaux utérins est bi-directionnel, et que les stéroïdes sont transportés entre les fœtus par diffusion à travers le liquide amniotique et les membranes fœtales des fœtus adjacents. Dans une autre étude, Even et ses collaborateurs (1992) ont implanté une capsule contenant de la testostérone tritiée dans la poche amniotique d'un fœtus aux jours 19 et 20 de la gestation ; ceci afin de déterminer la quantité de testostérone récupérée 12 heures plus tard au niveau du liquide amniotique des fœtus adjacents. Ces résultats supportent l'hypothèse que le transport, de la testostérone, et probablement aussi de l'œstradiol, entre les fœtus se produit au niveau des membranes fœtales par diffusion, de telle façon qu'un fœtus (mâle ou femelle) situé entre des fœtus mâles reçoit le plus fort apport de testostérone et qu'un fœtus (mâle ou femelle) situé entre des fœtus femelles reçoit le plus fort apport d'œstradiol.

B. Influence de la PIU sur le comportement

La majorité des études ont comparé des sujets 0M et 2M, mais chaque fois qu'un sujet 1M (qui se développe entre un fœtus mâle et un fœtus femelle) a été testé, il présentait des caractéristiques intermédiaires entre celles des sujets 0M et 2M. Jusqu'à aujourd'hui, les effets de la PIU ont été décrits chez les deux sexes et la plupart des paramètres observés concernaient les comportements reproducteur et agressif. L'influence de la PIU est différente selon les sexes, les phénotypes les plus susceptibles à la PIU sont le phénotype 2M, chez la femelle, et le phénotype 0M chez le mâle.

Chez la femelle, les sujets 2M montrent la distance ano-génitale la plus importante, c'est à dire que la distance entre l'anus et l'appareil génital est corrélée avec l'exposition prénatale à la testostérone (vom Saal et Bronson, 1978 ; Palanza et al., 1995). Ce type de femelle atteint plus tardivement la puberté et possède un cycle oestrien plus long et plus irrégulier (7 jours au lieu de 4) (vom Saal et Bronson 1980a ; 1980b ; vom Saal et al., 1981). Chez le rat et la souris, les femelles 2M et 0M diffèrent également dans leur capacité à attirer et à exciter les mâles. De plus, pendant l'œstrus, la femelle 2M est moins réceptive sexuellement (lordose moins intense) que la femelle 0M quet un mâle tente de la monter (Rines et vom Saal, 1984).

Chez le rat et la souris, la comparaison entre la capacité des femelles 2M et celle des femelles 0M à ovuler, à engendrer et à élever des petits a montré que les femelles 2M

cessent de mettre bas des portées vivantes à un âge significativement plus jeune et après moins de portées que les femelles 0M (vom Saal et Moyer, 1985 ; vom Saal, 1989). Chez la souris, elles montrent plus de comportement agressif envers les autres femelles (vom Saal et Bronson, 1978 ; vom Saal et al., 1983). L'agression inter-femelle ne semble pas être le résultat de l'établissement de dominance et influence significativement la reproductivité de la souris femelle. Kinsley et ses collaborateurs (1986a) ont montré que ce phénotype présente plus de comportement agressif vers un intrus mâle lors de la gestation et Hauser et Gnadelman (1983) ont observé une réduction des réponses d'évitement. Ainsi, chez les femelles, la proximité utérine avec des mâles est corrélée avec une diminution de la performance de reproduction et avec une augmentation du comportement agressif.

Chez les mâles, les souris et les rats 0M montrent plus d'intromissions que les sujets 2M lorsqu'ils sont placés avec une femelle sexuellement réceptive (vom Saal et al., 1983). Ainsi, chez les mâles, le développement utérin entre deux femelles est corrélé avec une augmentation de la performance sexuelle à l'âge adulte. Le phénotype 0M montre une augmentation de la tendance à l'infanticide et une diminution du comportement parental en présence d'un nouveau-né (vom Saal, 1984). Le fait que certains mâles souris commettent spontanément des infanticides lorsqu'ils mis en présence d'un petit a reçu plusieurs interprétations. Pour certains auteurs, il s'agit d'un comportement pathologique observé lors d'un stress social chez les rongeurs (voir Hrdy, 1979).

L'hypothèse de l'aromatisation dit que les fœtus qui sont exposés à des taux élevés d'œstradiol devraient être plus masculins que les fœtus exposés à des taux plus faibles de cette hormone. Cependant, les mâles souris 0M et 2M différent aussi dans leur réactivité à la thérapie de remplacement avec de la testostérone suite à une gonadectomie à la naissance. En fait, les sujets 0M requièrent une plus longue période d'exposition à la testostérone pour devenir agressif envers un autre mâle, et pour présenter une augmentation de la taille des vésicules séminales (Clemens et al., 1978, vom saal et al., 1983). Dans ce contexte, chez les mâles castrés à la naissance, le temps requis pour induire l'agression et le taux de croissance des vésicules séminales sont des indicateurs de la sensibilté à la testostérone. Comme ces données sont en opposition avec l'hypothèse d'aromatisation, il semble que, pour ces caractères, les taux élevés d'œstrogènes interfèrent avec l'action normale de sensibilisation de la testostérone pendant la vie fœtale par une action directe.

Ainsi, l'expression des comportements sexuel et agressif est positivement corrélée à l'exposition à la testostérone pendant la vie précoce (vom saal, 1979), alors qu'elle est négativement corrélée avec le comportement d'infanticide (Getelman et vom Saal, 1977 ; Samels et al., 1981). L'aire préoptique médiale de l'hypothalamus, qui est impliquée dans le comportement sexuel et probablement dans le comportement d'infanticide, est le centre

majeur d'aromatisation au sein des zones cérébrales exprimant les récepteurs aux œstrogènes (Kendrick et Drewett, 1980 ; Schleicher et al. , 1989).

Dans cette région, la testostérone est une pro hormone qui est aromatisée en œstradiol avant d'interagir avec les récepteurs aux œstrogènes intracytoplasmiques, ce complexe étant ensuite transporté dans le noyau de la cellule. Les taux élevés d'œstradiol observés dans le liquide amniotique des souris mâles 0M pourraient conduire à une augmentation du nombre de récepteurs aux œstrogènes activés qui sont transportés dans le noyau des cellules cibles, et entraîner une augmentation de la performance sexuelle à l'âge adulte. En effet, l'administration d'inhibiteur de l'aromatase , réduit le comportement sexuel chez les mâles (Christensen et al., 1975 ; Shinoda, 1994). Les autres régions hypothalamiques qui contiennent les récepteurs etrogènes (Luttge, 1979) pourraient être impliquées dans le comportement d'agression entre mâles. Dans ce cas, l'œstradiol pourrait se lier de façon compétitive aux récepteurs des etrogènes, et ainsi inhiber le comportement agressif

C. Signification adaptative de la position intra-utérine

La position intra-utérine et son influence sur la dynamique des populations

Le stress, dans le cas de portées surnuméraires et en réponse à l'augmentation du contact entre les animaux, influence la survie des individus ainsi que la performance de reproduction et, à plus long terme, la dynamique des populations chez les petits mammifères. Les souris, comme les autres rongeurs, peuvent subir des élévations de la taille des populations. Christian (1968), fut le premier auteur à montrer la corrélation positive existant entre la densité de la population et l'activité de l'axe HHS. Quet la densité de la population augmente rapidement, les interactions agressives augmentent : les animaux agressifs excluant de leur environnement les animaux les moins agressifs (Christian, 1970).

Etant donné que la PIU influence la capacité reproductrice et le comportement des individus, les changements dans la proportion des différentes PIU dans une population dues à la surpopulation peuvent influencer de façon marquée la dynamique des populations. Comme la densité de la population augmente, son comportement agressif élevé engendre la prédominance du phénotype 2M, et induit une disparition des animaux moins agressifs (0M et 1M). Etant donné que les animaux 2M ont à la fois un comportement plus agressif et une performance de reproduction plus faible, les interactions agressives chez les animaux 2M vont augmenter, ce qui aura pour conséquence une diminution de la taille de la population. Le profil des différences liées à la PIU suggère que, lorsque la densité de la population est faible, les souris femelles 0M peuvent avoir un avantage dans la reproduction sur les autres femelles car elles commencent leur puberté plus jeune et émettent les signaux les plus

attracteurs et les plus excitateurs pour les mâles.

Quet la densité de la population est élevée, les femelles souris 2M pourraient avoir un avantage reproducteur sur leur propre territoire car ce sont les plus agressives et qu'elles ont relativement insensibles aux signaux olfactifs inhibant la maturation sexuelle et la reproduction chez les souris (vom Saal et Bronson, 1978).

Le phénomène de la position intra-utérine est ainsi un modèle éthologique unique pour examiner les relations entre les taux des hormones sexuelles fœtales et le phénotype de l'adulte. Pour chaque sexe, la localisation utérine est un évènement dû au hasard (vom Saal, 1981) et il ne devrait donc pas y avoir de différences génotypiques systématiques entre les trois populations à PIU différentes dans d'une même portée. C'est pourquoi, les différences phénotypiques résultant de la PIU serait la conséquence de différences individuelles dans les concentrations hormonales plus que de différences génotypiques.

L'exposition au stress module l'influence de la position intra-utérine

Etant donné que la période de différentiation sexuelle chevauche l'ontogenèse de l'axe HHS, fonctionnel autour des jours 16-18 de gestation chez la souris et chez le rat (voir fig. X), il n'est pas surprenant que l'exposition d'une femelle gestante au stress, par contention ou par surpopulation, en plus des effets du stress maternel en lui-même, peut exercer une forte influence sur les différences physiologiques et comportementales de la descendance, celles-ci étant déjà influencées par la position intra-utérine.

Plusieurs études conduites dont le but était d'évaluer l'interaction entre le stress prénatal et la PIU chez la souris, ont démontré que chez les deux sexes, le stress maternel transforme les différences induites par une PIU entre deux femelles vers un profil 2M (vom Saal, 1983 ; Zielinski et al., 1991). Ainsi, les femelles 0M stressées prénatalement présentent une distance anogénitale plus grande, un cycle œstrien plus long (7 à 10 jours) et un comportement agressif plus important (vom Saal et al., 1990 ; 1991). D'un autre côté, les rats mâles 0M stressés prénatalement montrent une diminution de l'activité sexuelle, une réduction de la tendance à l'infanticide et un degré du comportement parental plus élevé.

L'élimination des différences de la PIU chez la descendance des souris stressées pourrait être due à une augmentation des taux circulants de testostérone chez les fœtus femelles (vom Saal et al., 1990) associé à une réduction des titres en œstrogènes circulants dues à une réduction de l'activité aromatase chez les deux sexes (Weisz et al., 1982). Le stress maternel augmente la concentration de testostérone chez les fœtus mâles au jour 17 de gestation et la diminue fortement au jour 18, jour critique développement prénatal pour la masculinisation du comportement sexuel des mâles par la testostérone (Ward et Weisz, 1980). Après l'exposition au stress, l'hormone corticotrope (ACTH) maternelle est libérée et

stimule la libération de corticostérone par les surrénales de la mère. La corticostérone traverse la barrière placentaire et diminue la sécrétion fœtale d'ACTH hypophysaire, conduisant à une diminution de la sécrétion des stéroïdes par les surrénales du fœtus. Cependant, la plus forte réduction de la biosynthèse en œstradiol pourrait être observée chez les fœtus femelles, entraînant l'absence de différences en œstradiol lors de la vie prénatale chez tous les fœtus entourés de fœtus femelles (individus 0M).

II.LE PHENOMENE DU STRESS IN UTERO

De nombreuses études épidémiologiques menées chez la femme ont montré les effets dramatiques du stress lors de la grossesse : avortement spontané, accouchement difficile, faible poids du nouveau-né à la naissance, pourcentage élevé d'enfants prématurés, augmentation de pathologies néonatales, retard de développement et altérations comportementales à long terme (Stott, 1973; Blomberg, 1980; Meijer, 1985; Homer et al., 1990; pour revue, voir Weinstock, 2001). A l'heure actuelle, les mécanismes sous-tendant les effets du stress prénatal (SP) ne sont pas complètement établis. Cependant, les taux élevés de glucocorticoïdes maternels lors de la réponse au stress pourraient être des cetidats de choix dans la genèse des troubles liés au SP.

Des études menées chez l'Homme ont montré que les concentrations de cortisol maternel et fœtal sont positivement corrélées (Gitau et al., 1998), que l'exposition prénatale au glucocorticoïdes diminue le poids du nouveau-né à la naissance (pour revue, voir Seckl, 2001), et que l'administration prénatale de faibles doses d'un glucocorticoïde de synthèse (la déxaméthasone) modifie les caractéristiques neuropsychologiques de l'individu, telles que son émotivité (Trautman et al., 1995; Lajic et al., 1998). Chez le rat, l'exposition prénatale à la dexaméthasone réduit le poids à la naissance, altère le développement cérébral (Slotkin et al., 1993) et programme l'hypertension et l'hyperglycémie à l'âge l'adulte (pour revue, voir Nyirenda et al., 1998).

Si on supprime la libération de corticostérone maternelle lors d'un stress et que l'on normalise les taux de glucocorticoïdes maternels par une surrénalectomie suivie d'une thérapie de substitution de la corticostérone, on inhibe l'augmentation de la réponse de l'axe HHS et la diminution des récepteurs aux glucocorticoïdes, habituellement observées chez les rats PS (Barbazanges et al., 1996b). Ces résultats suggèrent, pour la première fois, que l'augmentation des glucocorticoïdes maternels induite par le stress altère le développement de la fonction hypothalamo-hypophyso-surrénalienne chez la descendance. Chez le fœtus, les taux de cortisol (corticostérone chez les rongeurs) sont plus faibles que chez la mère (Beitens et al., 1973). En effet, l'enzyme 11 ß hydroxystéroide deshydrogenase (11ß-HSD2, Brown et al., 1996) qui est hautement exprimée dans le placenta et dans de nombreux tissus fœtaux jusqu'à la moitié de la gestation, catalyse la conversion du cortisol (ou corticostérone) en cortisone (11-dehydrocorticosterone). Des individus homozygotes pour des mutations délétères au niveau du gène de la 11ß-HSD2 montrent un faible poids à la naissance (White et al., 1997). De plus, chez le rat, les variations interindividuelles de l'activité de la 11ß-HSD2 sont positivement corrélées avec le poids à la naissance (Benediktsson et al. 1993; 1997). Des résultats similaires ont pu être observés chez l'homme (Stewart et al., 1995). Chez le rat, plusieurs études ont montré que l'inhibition de la 11ß-HSD2 placentaire par

l'administration de carbenoxolone à des femelles gestantes, diminue le poids du nouveau–né à la naissance, programme l'hypertension et l'hyperglycémie à l'âge adulte (Lindsay et al., 1996) et engendre des altérations permanentes de l'axe HHS et du comportement anxieux dans des situations aversives (Welberg et al., 2000).

Les difficultés éthiques et les limites des études rétrospectives, inhérentes à la recherche chez l'homme, ont amené la communauté scientifique à appréhender les effets du stress prénatal de façon plus approfondie à l'aide de modèles animaux, et plus particulièrement chez le rat. Diverses procédures ont ainsi été adoptées dans le but de soumettre la femelle gestante à des conditions de stress (pour revue voir Weinstock, 2001). Les plus fréquemment utilisées sont le bruit combiné à des flashs lumineux, appliqués de façon aléatoire trois fois par semaine lors de la gestation (Fride et Weinstock, 1984), ou le stress de contention trois fois par jour durant la dernière semaine de gestation (Ward et Weisz, 1984; Maccari et al., 1995; Alonso et al., 1991), période durant laquelle l'axe HHS du fœtus commence à libérer de l'ACTH et de la corticostérone fœtales (Bodouresque et al., 1988). Ce dernier paradigme a été adopté dans la majorité des travaux décrits dans ce chapitre, ainsi que dans les quatre études qui font l'objet de cette thèse (chapitres 2, 3 et 4).

A. Effets du Stress Prénatal sur la physiologie

Pour être efficace, la réponse au stress de l'axe HHS doit être rapide et brève. Un échec de le rétablissement des taux circulants de glucocorticoïdes à leur niveau de base augmente le risque d'une altération permanente du rétrocontrôle de l'axe HHS.

La régulation de l'axe HHS s'opère au niveau des récepteurs aux glucocorticoïdes (GRs) et des récepteurs aux minerallocorticoïdes (MRs) par un rétrocontrôle négatif phasique et par une influence inhibitrice tonique (de Kloet et Reul, 1987; De Kloet et al., 1998). Les GRs, largement distribués au niveau des étages hypothalamique et hypophysaire de l'axe HHS, permettent la régulation des élévations phasiques de l'axe HHS : le rythme circadien et la réponse au stress (Reul et al., 1987; pour revue voir De Kloet et al., 1998). Quant aux MRs, principalement localisés dans l'hippocampe, ils exercent une action inhibitrice tonique (Gerlach et McEwen, 1972; Reul et De Kloet, 1985).

Chez le rat, le stress maternel induit, de façon évidente, une régulation anormale de l'axe HHS chez la descendance. En effet, chez des rats non sevrés, le SP augmente la sécrétion de corticostérone lors d'un stress (Henry, 1994). De plus, chez les animaux adultes SP, la sécrétion de corticostérone est prolongée après un stress (Fride et al. 1986). Plusieurs études ont également montré que les animaux adultes SP présentent une diminution de la capacité de liaison aux MRs et aux GRs hippocampiques (Weinstock et al., 1992; Henry et al., 1994; Maccari et al., 1995; Barbazanges et al. 1996b; Koehl et al., 1997). En effet la

hausse des taux de glucocorticoïdes circulants lors du vieillissement est potentialisée par le SP (pour une revue sur le vieillissement et l'axe HHS, voir Sapolsky, 1992). Ainsi, des rats d'âge moyen présentent une élévation de la sécrétion basale de corticostérone, qui devient alors comparable à celle des rats âgés (Vallee et al., 1999).

Bien que ces résultats montrent une altération du rétrocontrôle de l'axe HHS chez les rats SP, ils suggèrent que les variations des récepteurs aux corticoïdes ne causent pas l'élévation des glucocorticoïdes circulants, mais lui sont consécutives.

Ce phénomène pourrait être sous-tendu par une anomalie des processus d'adaptation normaux décrits plus haut ; en effet, suite à un stress, des rats âgés de 3 jours ont des taux de corticostérone plasmatique plus élevés que des nouveaux-nés contrôles, bien qu'ils aient le même nombre de récepteurs aux corticostéroïdes hippocampiques (Henry et al., 1994).

Chez le rat, le SP affecte le système sérotoninergique, en augmentant les taux cérébraux de 5-HTau niveau du cortex ou en les diminuant au niveau de l'hippocampe (Peters, 1988; 1990). Les changements au sein de la fonction sérotoninergique pourraient être impliqués dans les modifications observées au niveau de l'axe HHS, étant donné qu'il existe une influence réciproque entre ces deux systèmes (Joels et al., 1991; De Kloet et al., 1998). De plus, une augmentation de la libération d'acétylcholine hippocampique suite à un stress ou à l'injection de corticoliberine (CRH) démontre les effets à long terme sur le développement des systèmes cholinergiques du cerveau antérieur (Day et al., 1998). De plus, la CRH est augmentée au niveau de l'amygdale (Cratty et al., 1995). Le SP engendre également une diminution des taux et du renouvellement de la noradrénaline et de la dopamine au niveau cérébral (Fride et Weinstock, 1988; Takahashi et al., 1992; Henry et al., 1995). Dans ce contexte, plusieurs études ont montré que, chez le rat mâle, le SP élimine les asymétries de la fonction dopaminergique striatale et diminue les asymétries de la taille du cortex cérébral (Alonso et al., 1991; 1994; 1997; Fleming et al., 1986). Comme chez l'homme, les rats normaux présentent des asymétries cérébrales qui sont liées à l'organisation du cerveau et qui contrôlent de nombreux comportements (Carlson et Glick, 1989). Ces modifications spécifiques induites par le stress gestationel peuvent être mise en parallèle des observations cliniques démontrant une réduction des asymétries cérébrales (pour revue, voir Weinstock, 2001). Les changements observés au niveau du système dopaminergique ont des implications importantes dans le développement d'une sensibilité accrue aux psychostimulants chez ces animaux (Deminiere et al., 1992; Henry et al., 1995; Koehl et al, 2000).

La question de la vulnérabilité aux drogues chez les rats SP est discutée dans la prochaine section et constitue le point central de l'étude décrite dans le chapitre 2 de ce travail de thèse.

B. Effets du Stress Prénatal sur le comportement

L'exposition au stress prénatal, chez l'homme comme chez l' animal, induit des effets à long terme sur le comportement, résultant en une altération des capacités adaptatives de l'individu (pour revue, voir Weinstock, 2001).

Chez l'animal, le stress durant la gestation interrompt le cours normal de la différentiation sexuelle en diminuant les taux de testostérone chez le rat mâle à la naissance (Ward, 1972), ce qui a pour conséquence une altération de la fonction sexuelle (Masterpasqua et al., 1976; Ward, 1983). Des modifications dans le développement moteur précoce ont été décrites chez les animaux SP (Barlow, 1978). Chez le mâle, le SP réduit le comportement de jeu, le ramenant au niveau de celui des femelles (Ward et Stehm, 1991), et ainsi éliminant les différences sexuelles normalement observées dans ce comportement (Meaney, 1989). Les singes SP présentent une diminution du comportement de jeu et de l'exploration comparés à des animaux contrôles, cette diminution est associée à une élévation du comportement d' "agrippement" aux autres singes. Ce comportement est normalement associé aux parents ; le fait qu'il soit pratiquée sur d'autres congénères témoigne d'une plus grande anxiété face à la nouveauté. Ce résultat a aussi été confirmé chez l'homme par Meijer (1985) qui a montré que les enfants SP ont une activité ludique réduite et sont moins sociables que les autres enfants.

Chez les animaux normaux, la relation entre l'activité et la peur en présence de situations génératrices de peur a la forme d'une fonction en U inversé. Cependant, le SP, chez les rats adultes (Ward et Weisz, 1984; Wakshlask et Weinstock, 1990; Poltyrev et al., 1996; Vallee et al., 1997) et chez les singes (Schneider, 1992), engendre moins d'exploration et plus de défécation et de comportement de fuite que les animaux contrôles dans un nouvel environnement. De telles différences ont pu être abolies par un traitement aux anxiolytiques, tels que les benzodiazépines (Pohorecky et Roberts, 1991; Drago et al., 1999). Cependant, Vallee et ses collaborateurs (1997) ont démontré que les effets du SP sur plusieurs composantes du comportement émotionnel sont fortement corrélés avec les taux plasmatiques de corticostérone en réponse à un stress. En conclusion, les altérations de l'axe HHS induites par le SP pourraient sous-tendre les changements de la réactivité comportementale chez l'adulte.

C. Effets du Stress Prénatal sur les rythmes circadiens

La majorité des processus physiologiques ou comportementaux fluctuent au cours des 24 heures d'une journée. Ce rythme résulte d'un système interne, l'horloge circadienne, située au niveau des noyaux suprachiasmatiques de l'hypothalamus (Stephan et Zucker, 1972; Turek et Van Reeth, 1995).

En l'absence d'influx environnementaux, ces rythmes persistent avec une périodicité de 24 heures environ et sont nommés, en conséquence, rythmes circadiens. Les changements de cycle lumière / obscurité (Pittendrigh, 1981), mais aussi des stimuli neurochimiques et comportementaux (Van Reeth et Turek, 1989) influencent la fonction circadienne et les profils de sommeil. Parmi ces stimuli, les stéroïdes ont un effet marqué sur le fonctionnement du système circadien (Turek et Gwinner, 1986). Chez le rat adulte, le stress chronique peut induire des changements des rythmes circadiens et des profils de sommeil (Kant et al., 1995 ; Cespuglio et al., 1995). Ainsi, chez le rat, le SP induit une avance de phase de la sécrétion de corticostérone, avec des niveaux plus hauts de corticostérone total et libre secrétée à la fin de la période lumineuse dans les deux sexes et une sécrétion de corticostérone augmentée sur la totalité du cycle diurne chez la femelle (Koehl et al., 1997 ; 1999). Les effets du SP sur le rythme de sécrétion de la corticostérone pourraient être dus à la diminution de l'expression des récepteurs aux corticostéroïdes hippocampiques à des moments spécifiques de la journée. En effet, une diminution des MRs au début de la phase lumineuse a pu être observée chez le mâle, et à la fin de la phase lumineuse chez le mâle et chez la femelle (Koehl et al., 1999). Le SP entraîne une altération de la fonction temporelle de l'axe HHS, renforçant l'idée d'un dysfonctionnement homéostatique général chez ces animaux.

Les troubles du rythme circadien de l'activité locomotrice ont également pu être démontré chez les animaux SP. Ceux-ci incluent une diminution des taux de resynchronisation de l'activité rythmique qui survient après un changement abrupt du cycle lumière / obscurité (Van Reeth et al., 1998) et une avance de phase dans le rythme de comportement locomoteur dans une roue (« *wheel running* ») (Koehl et al., 1999).

Une autre modification importante des rythmes circadiens, induite par le SP, concerne les changements dans le profil veille / sommeil qui sont observés chez l'animal adulte (Dugovic et al., 1999). En conditions de base, les rats SP montrent une augmentation de la quantité de sommeil paradoxal. Cette augmentation est positivement corrélée au taux de corticostérone plasmatique. Le SP engendre également une augmentation de la fragmentation du sommeil, une augmentation du temps de sommeil à ondes lentes et une faible diminution du pourcentage de sommeil lent profond relativement au temps total de sommeil. Pendant la période de sommeil réparateur consécutive à un stress aigu de

contention, tous les changements de sommeils persistent et sont corrélés avec la sécrétion de corticostérone induite par le stress. Des taux élevés de corticostérone en condition de base tout comme lors d'un stress aigu pourraient prédire des altérations à long terme des cycles veille / sommeil. Ajoutés aux glucocorticoïdes, d'autres facteurs pourraient être impliqués dans les effets à long terme du SP sur le sommeil. Ainsi, le système sérotoninergique pourrait faire partie de ces facteurs. L'exposition à des niveaux élevés de glucocorticoïdes ou à des stresseurs aigus engendrent des altérations significatives du renouvellement de la 5-HT dans la région mésencéphalique / pontique chez le rat SP (Muneoka et al., 1997). Comme il a été précédemment montré, chez le rat, le SP engendre une altération à long terme de la réponse aux agonistes des récepteurs sérotoninergiques (Peters, 1988). Vu le rôle permissif joué par le système sérotoninergique sur la régulation du sommeil paradoxal (voir Boutrel et al., 1999 ; Dugovic et al., 1989 ; Jouvet, 1969) et sur la modulation du cycle veille / sommeil, des altérations développementales du métabolisme cérébral de la 5-HT pourraient contribuer à la modification des paramètres du sommeil induites par le SP.

La modification des rythmes circadiens et le dérèglement global de l'activité de l'axe HHS observé chez le rat SP, peuvent être mis en parallèle avec les troubles du rythme circadien observés chez la majorité des patients dépressifs (Rosenwasser et Wirz-Justice, 1997). Cela suggère que les rats SP pourraient être un modèle animal adéquat de dépression. Ce dernier point fait l'objet de la section suivante et constitue le point central de deux études du chapitre 3 de cette thèse.

III. LES MODELES ANIMAUX DE PSYCHOPATHOLOGIES

Dans les sections précédentes, nous avons vu que des modifications subtiles ou profondes de l'action d'organisation des hormones sexuelles ou des glucocorticoïdes pouvaient affecter le développement normal et, en conséquence, exercer un effet à long terme sur les capacités adaptatives d'un individu. Dans cette section, nous avons considéré les facteurs programmant la vulnérabilité à l'abus de drogues et aux troubles similaires à la dépression dans les modèles animaux.

A. L'abus de drogue

Les modèles animaux de l'abus de drogues ont été indispensables dans les stratégies de développement de la prévention et des traitement utilisés en clinique. Les études expérimentales et cliniques ont montré qu'un des principaux facteurs conditionnant le développement de l'addiction est une sensibilité particulière aux effets renforçant de l'abus de drogue chez certains individus (De Wit et aL, 1986). Plusieurs travaux ont démontré que les différences sexuelles, l'exposition à des situations stressantes et/ou le stade de développement du sujet, jouent un rôle important dans la détermination d'une telle variabilité interindividuelle.

1. Les différences sexuelles et la sensibilité aux drogues

La dépendance et l'abus de drogues diffèrent entre les hommes et les femmes. Des différences dans la sensibilité aux drogues et à l'auto-administration ont également été observées chez des animaux de laboratoire. Par exemple, les femelles apparaissent plus vulnérables que les mâles aux effets renforçateurs des psychostimulants, des opiacées et de la nicotine lors de plusieurs phases du processus d'addiction (pour revue, voir Lynch et al., 2002).

Plusieurs données suggèrent que les différences sexuelles dans la prévalence de la consommation de drogues puissent être du aux différences d'opportunité de consommation de drogues plus qu'à une vulnérabilité à la consommation de drogues. De façon spécifique, Van Etten et ses collaborateurs (1999) , ont comparé l'occurrence des opportunités de consommer de la marijuana, de la cocaïne et de l'héroïne entre des adolescents, filles et garçons.

Ces données suggèrent que les hormones gonadiques sont importantes dans la réponse aux drogues. Parmi les nombreuses études conduites sur ce sujet (pour revue voir Lynch et al., 2002), nous limiterons notre rapport à quelques données obtenues sur l'action des hormones sexuelles sur le système μ opioïdergique en insistant sur le lien entre le

développement temporel de ces récepteurs et la différenciation sexuelle de l'individu. En effet, les peptides opiacées et les récepteurs mu sont déjà exprimés au jour 17 de gestation alors que d'autres récepteurs tels que les récepteurs delta et kappa apparaissent principalement lors de la période post natale (De Vries et al., 1990; Rius et al., 1991).

De plus, des profils de prise d'opiacés ont été comparés entre des animaux mâles et des animaux femelles. Plusieurs études ont démontré l'absence de différences sexuelles dans les taux d'auto-administration d'héroïne lors de la session initiale d'auto-administration de la drogue. Par contre, lorsque les rongeurs mâles et femelles sont comparés sous soit des conditions étendues d'accès soit sur une longue période, les souris et les rats femelles consomment des taux plus élevés d'héroïne (Carrol et al., 2001) et de morphine (Alekseter et al.,1978 ; Hadaway et al., 1979) que les mâles. Ces résultats suggèrent que les différences sexuelles dans la prise d'opiacés pourraient dépendre des conditions d'accès et que cela devienne plus apparent avec le temps.

Au niveau comportemental, des différences sexuelles dans la douleur modulent l'efficacité des analgésiques opïoides (Tershner et al., 2000). En effet, les mâles présentent un seuil de douleur plus élevé et une analgésie induite par la morphine plus importante que les femelles (Beatty 1979 ; Forman et al. 1989 ; Kavaliers et Colwell 1991). Les animaux exposés à la testostérone dans la période néonatale sont moins sensibles aux effets des drogues psychoactives sur l'activité locomotrice, alors que chez les rats femelles les effets de la morphine sont augmentés par l'œstradiol circulant (Forgie et Stewart 1993 ; Stewart et Rodaros 1999).

La densité des récepteurs mu dans l'hypothalamus est plus élevée chez les femelles que chez les mâles (Hammer, 1988). Ce profil de maturation peut être annulé par la castration néonatale des mâles et par un traitement à la testostérone des femelles, indiquant ainsi qu'il est lié à la présence de testostérone à la naissance, au moins pour cette région (Limonta et al. , 1991 ; Maggi et al., 1991). De plus, chez les rongeurs et chez les primates non humains, la testostérone est reconnue pour réguler l'expression du gène de la pro-opiomélanocortine dans le noyau arqué de l'hypothalamus, probablement après sa conversion en œstradiol (Adams et al. 1991).

2. Influence du stress sur la vulnérabilité aux drogues

Le modèle animal traditionnel implique l'entraînement d'un animal à s'auto-administrer une dose relativement élevée d'une drogue en utilisant équitablement des conditions d'accès réduites lors de sessions quotidiennes brèves. Dans ces conditions, beaucoup d'animaux s'auto-administrent de la drogue. A cet égard, les animaux de laboratoire présentent des différences interindividuelles importantes dans l'auto-administration d'amphétamine car, à

des doses faibles, seulement quelques rats s'auto-administrent de la drogue (Piazza et al., 1989).

La principale caractéristique de ces animaux était un déséquilibre dans l'activité dopaminergique mésolimbocorticale reflétée par une plus grande activité dopaminergique dans le noyau accumbens et une activité plus faible dans le cortex préfrontal. Ils présentaient également une plus grande réactivité comportementale et endocrinienne au stress, caractérisée par une plus grande réactivité locomotrice et une sécrétion de corticostérone en réponse à la nouveauté (Piazza et al., 1990, 1991). La sécrétion de corticostérone prolongée chez ces animaux est due à une plus faible affinité des récepteurs aux corticoïdes hippocampiques (Maccari et al., 1991). Finalement, ces animaux montrent une activité locomotrice plus élevée consécutivement à l'administration de psychostimulants (Hooks et al., 1991). D'autres études ont établi que les expositions répétées d'animaux adultes à des conditions stressantes augmentent la vulnérabilité individuelle aux psychostimulants (Deroche et al., 1992 ; 1993 ; pour revue voir Piazza et Le Moal, 1996)

Chez les rats, l'exposition au stress lors de la vie prénatale affecte les systèmes pharmacologiques qui sont particulièrement importants dans l'étude de l'abus de drogues. En effet, le SP entraîne des altérations fonctionnelles du système mésolimbique conduisant à une élévation de la densité des récepteurs dopaminergique D2 et à une diminution des récepteurs dopaminergiques D3 dans le noyau accumbens de la descendance adulte. Le contrôle altéré de la sécrétion de corticostérone observé chez ces animaux est un cetidat potentiel comme intermédiaire des effets du stress maternel sur le système dopaminergique de la descendance. Les glucocorticoïdes favorisent la sensibilisation des rats aux amphétamines chez les rats principalement grâce aux GRs (Rivet et al., 1989), alors que l'administration de psychostimulants active l'axe HHS. De plus, le blocage de l'axe HHS atténue la sensibilisation induite par les amphétamines sans modifier l'amplitude de la première injection d'amphétamine (Cole et al., 1990). Notons que les taux circulants de corticostérone sont corrélés avec une propension augmentée d'auto-administration d'amphétamine (Piazza et al., 1991). Cette idée est supportée par la présence de récepteurs aux glucocorticoïdes dans les neurones dopaminergiques de l'aire tegmentale ventrale, projetant sur le noyau accumbens et par l'observation que les glucocorticoïdes modulent la libération de dopamine dans le système mésolimbique (Harfstret et al., 1986 ; Piazza et Le Moal, 1997).

En accord avec le profil neurochimique déjà explicité, les rats SP présentent une propension augmentée à développer de l'auto-administration d'amphétamine, une sensibilté augmentée aux effets locomoteurs de la drogue et aussi une plus grande réponse comportementale à la nicotine. Ces études ont souligné l'importance de l'environnement précoce dans le développement ultérieur du comportement associé aux drogues.

3. L'âge du sujet, un facteur important dans la détermination de la variabilité individuelle

Lors de l'adolescence, le risque d'abus de drogues augmente (pour revue, voir Spear, 2000). Lors de l'ontogenèse, les systèmes cérébral et hormonal sous-tendent encore les réarrangements de la maturation, qui se met en place en parallèle avec des modifications significatives du développement psychosocial. Chez l'homme, les adolescents montrent une quantité disproportionnée de comportement d'insouciance, de recherche de sensation et de prise de risque, comparé aux individus d'âges différents. Les niveaux élevés de recherche de nouveauté et de sensation sont des prédicteurs puissants de la consommation de drogue et d'alcool (Etrucci et al., 1989 ; Wills et al., 1994). Le modèle animal de l'adolescence chez le rat (validé par Spear et Brake, 1983 ; Laviola et al., 1999) représente un outil pratique pour l'observation de la vulnérabilité à des agents variables entraînant une habitude ou des expériences émotionnelles dont les propriétés renforçatrices pourraient être liées des substrats neurobiologiques communs.

Les rats et les souris adolescents montrent des niveaux de base de locomotion et d'activité exploratrice élevés comparé à leurs congénères plus jeunes ou plus âgés (Adriani et al., 1998). Les sujets periadolescents sont caractérisés par de meilleures performances dan des paradigmes expérimentaux qui requiert que l'animal émette une réponse locomotrice simple dan le but d'obtenir un renforcement et montrent une altération dans les tests d'apprentissage qui requièrent que l'animal accorde une attention particulière aux signaux environnementaux (Spear et Brake, 1983). Chez les animaux, les adolescents d'une grande variété d'espèces sont différents des adultes dans leur sensibilité psychopharmacologique aux stimulants locomoteurs (Spear, 2000) et à l'alcool (Little et al., 1996 ; Silveri et Spear, 1998). Les rats et les souris adolescents sont moins sensibles que les adultes à l'amphétamine et à la cocaïne lorsqu'on observe les paramètres de stimulation locomotrice et de stéréotypie (Spear et Brick, 1979 ; Bolanos et al., 1998 ; Laviola et al., 1999 ; Spear, 2000). Cette sensibilité réduite à l'amphétamine lors de l'adolescence n'apparaît pas comme étant simplement due à une altération des niveaux de drogues cérébraux liée à l'âge (Spear et Brake, 1983) et ceci est encore valable lorsqu'on examine les aversions de goût conditionnées induites par l'amphétamine (Infurn et Spear, 1979). De façon intéressante, les animaux adolescents développent une sensibilisation locomotrice aux effets locomoteurs de la cocaïne et de l'amphétamine lorsqu'ils y ont été exposés de façon chronique lors de l'adolescence mais on n'observe pas de sensibilisation des effets stéréotypés comme cela est observé chez les adultes (Adriani et al., 1998). L'exposition à la drogue lors de l'adolescence peut avoir une influence à long terme sur les fonctions neurocomportementales ultérieures. Une consommation volontaire d'éthanol (Salimov et al.,

1996) ou une consommation chronique de cocaïne (Harrison et al., 1997) affecte significativement le comportement émotionnel chez le rat et le comportement agressif chez le hamster, alors qu'une exposition répétée et intermittente au MDMA lors de l'adolescence augmente les comportements exploratoires et sociaux chez la souris (Morley-Fletcher et al., 2002).

Les différences de développement dans la sensibilité psychopharmacologiques entre les adolescents et les adultes pourrait également être liées aux changements ontogénétiques du fonctionnement des substrats neuronaux sur lesquels ces drogues agissent. En effet, le cerveau adolescent diffère considérablement du cerveau adulte au niveau d'un gret nombre de systèmes neuraux impliqués dans l'action de ces drogues (Etersen et al., 1997 ; Spear, 2000). Seeman et ses collaborateurs ont observé, chez l'homme, des changements notables dans les populations de récepteurs DA dans le striatum lors de la période juvénile avec un tiers à la moitié ou plus de récepteurs D1 et D2 présents dans le striatum des juvéniles et absents dans le cerveau adulte (Seeman et al., 1987). Chez les rats, de manière identique, la liaison aux récepteurs D1 et D2 dans le striatum subirait un déclin lors du développement, montrant un pic à l'adolescence à des niveaux supérieurs de 30-45% à ceux observés chez l'adulte (Teicher et Etersen, 1995). De fortes évidences supportent également la suggestion que le système 5-HT subit des altérations en fonction du temps. Dillon et ses collaborateurs (1991) ont reporté une corrélation négative entre l'âge et la liaison au récepteur 5-HT1A dans des cerveaux humains masculins en post mortem. Chez le rat, l'innervation sérotoninergique du cerveau antérieur basal montre des variations considérables en fonction de l'ontogenèse, avec un nombre de synapses sérotoninergiques atteignant les niveaux des adultes au j14 et diminuant de façon considérable en dessous des niveaux adultes au sevrage, au jour 21 (Dinopoulous et al., 1997).

En regard de ces mécanismes, ces différences ontogénétiques dans la sensibilité aux drogues pourraient avoir des conséquences significatives pour l'adolescent. Etant donné que, les sujets adolescents montrent une sensibilité réduite à l'abus de drogues variées, une telle insensibilité peut entraîner une consommation par prise supérieure à celle d'individus plus matures.

Une partie de ce travail de thèse, le chapitre 1, a été consacrée à l'évaluation des effets exercés par deux facteurs de programmation, les hormones sexuelles et les glucocorticoïdes, sur la vulnérabilité individuelle à l'abus de drogue chez le rat adolescent. Deux modèles de variation d'exposition à ces hormones ont été considérés : 1)un modèle naturel tel que la position intra-utérine chez la souris et 2) un modèle induit par le stress tel que le stress prénatal chez le rat.

B. Les troubles dépressifs

Les troubles dépressifs font partie des pathologies humaines les plus fréquentes : environ 11% des individus subissent un épisode de dépression au moins une fois dans leur vie (Judd, 1995). La dépression est caractérisée par différents symptômes, qui se manifestent avec une fréquence et une chronicité (plus de deux semaines) suffisantes pour constituer une entité clinique identifiable. Les caractéristiques majeures de la dépression sont (i) un manque de motivation et une incapacité à éprouver du plaisir (anhédonie), (ii) une perte d'appétit, (iii) des troubles du sommeil (augmentation de la quantité de sommeil paradoxal et diminution de sa latence) (iv) ralentissement psychomoteur ou agitation, (v) un changement de phase des rythmes circadiens (Whybrow et al., 1984 ; Yadid et al., 2000). Une variabilité de méthodes (pharmacologie, psychothérapie, thérapies par électrochocs et magnétiques) peuvent être utilisées pour traiter efficacement la dépression, mais leur succès reste limité (Nestler, 1998 ; Stahl, 1998 ; Ressler et Nemeroff, 1999).

L'altération de l'axe HHS est parmi les caractéristiques biologiques les plus cohérentes, au moins chez les sous-populations de sujets déprimés. Ceci est caractérisé par une hypersécrétion de cortisol (Sackar et al., 1973 ; Holsboer et al., 1983 ; Rubin et al., 1987), une résistance au test de la déxaméthasone (Arana et Mossman, 1988), une élévation des taux de corticolibérine (CRF) dans le liquide cérébro-spinal (Nemeroff et a., 1984), une diminution de la liaison aux récepteurs du CRF au niveau du cortex préfrontal (Nemeroff et al., 1988), et une hyperactivité du système cholinergique (Janowsky et al., 1980). Une dysfonction générale du système sérotoninergique (5-HT) a également été reportée. Une diminution de la capture de la 5-HT et une altération des niveaux post synaptiques des récepteurs 5-HT, tels que les récepteurs 5-HT1A et 5-HT2 dans le cortex et dans l'hippocampe (Lesch et al., 1991 ; Lanfumey et al., 2000) pourraient indiquer un mécanisme compensatoire d'une activité sérotoninergique trop faible qui caractérise les patients dépressifs (Meltzer et Lowy, 1987 ; Maes et Meltzer, 1995 ; Feldman et al., 1997). Une augmentation de la disponibilité synaptique de sérotonine causée par les antidépresseurs supporte cette théorie (Blier et al., 1990). Les troubles du sommeil observés chez les patients dépressifs sont également en accord avec la théorie sérotoninergique, car une augmentation de la disponibilité en 5-HT dans les synapses résultant du blocage de la recapture de la sérotonine peut engendrer une diminution de la fréquence du sommeil REM (Meltzer et Lowy, 1987 ; Maes et Meltzer, 1995 ; Feldman et al., 1997). Des événements de vis stressants font partie des facteurs environnementaux potentiels qui pourraient provoquer des épisodes dépressifs (Paykel, 1978 ; Anisman et Zacharko, 1982 ; Sapolsky, 1996 ; Glover, 1997 ; Holsboer, 2001).

L'hypothèse du stress dans les troubles de l'humeur a conduit à la suggestion que les modèles de dépression induits par le stress ont une "construct validity" (cohérence théorique) (Rosenwasser et Wirz-Justice, 1997 ; Willner, 1997). Les modèles animaux des troubles psychiatriques tels que la dépression peuvent être évalués par leur cohésion à trois critères majeurs. Le premier critère est la "face validity", qui évalue l'homologie des symptômes du modèle avec ceux décrits chez l'homme. Le second critère est la "predictive validity", qui examine la question de la sensibilité aux traitements qui affectent ou non les symptômes décrits chez l'homme. Le troisième critère est la "construct validity" qui évalue la cohérence du modèle avec les hypothèses théoriques (Willner, 1991). Parmi les modèles animaux de dépression qui ont été développés (pour revue, voir Yadid et al., 2000), nous avons choisi le modèle du SP chez le rat. Jusqu'à aujourd'hui, les études conduites dans nos groupes et dans d'autres groupes indiquent que la "face validity" du modèle de SP est élevée car plusieurs anormalités peuvent être mises en parallèle avec celles trouvées chez les patients dépressifs.

1. L'intérêt du Stress Prénatal chez le rat comme modèle animal de la dépression

Les rats SP présentent une altération du rétrocontrôle négatif de l'axe HHS (Henry et al., 1994 ; Maccari et al., 1995 ; Barbazanges et al., 1996 ; Koehl et al., 1997 ; 1999), une augmentation des taux de CRH dans l'amygdale (Cratty et al., 1995) et dans l'éminence médiane (Smythe et al., 1996), et une hypersensibilité à l'injection de CRH (Day et al., 1998). En accord avec les dysfonctions observées dans le système sérotoninergique chez les patients déprimés (Meltzer et Lowy, 1987), les rats SP montrent des taux de récepteurs 5-HT2 post synaptiques augmentés (Peters, 1986 ; 1988 ; 1990).

En concordance avec les altérations de la régulation du cycle veille-sommeil et les augmentations du sommeil paradoxal reportées chez l'humain est une marque de la dépression (Kupfer et Reynolds, 1992 ; Polet et al., 1992), les rats SP adultes présentent des changements persistants dans l'architecture du sommeil, comparables à celles trouvées chez les patients dépressifs (Dugovic et al., 1999). De plus, les corrélations significatives entre les anormalités du sommeil et la dysfonction de l'axe HHS ont été observées chez les patients dépressifs (Polet et al., 1992 ; Hubain et al., 1998) et pourraient résulter du stress (Rosenwasser et Wirz-Justice, 1997). Dans ce contexte, il est important de comprendre que la persistance des altérations du sommeil paradoxal observées chez les patients dépressifs est totalement différente des anormalités temporaires du sommeil observées dans d'autres modèles de stress tels que le modèle de stress chronique léger (Cheeta et al., 1997 : Moreau et al., 1995), dans lequel le sommeil paradoxal est augmenté seulement lors du premier jour de récupération parés le stress ou disparaît très vite après la fin du stress. D'un

autre côté, le SP induit une réduction de la durée de vie de la neurogenèse dans l'hippocampe (Lemaire et al., 2000). Ceci est en accord avec le remodelage structural induit par le stress au sein de l'hippocampe qui peut caractériser la détérioration de la plasticité neuronale dans le cerveau humain lors des troubles dépressifs (Sheline et al., 1996 ; Sapolski, 2000).

D'un point de vue comportemental, les rats SP montrent un comportement anxieux (Vallee et al., 1997 ; Weinstock et al., 2001) et une co-morbidité avec l'anxiété qui a été observée chez les patients dépressifs (Stahl, 1993 ; Rouillon, 1999).De plus, Alonso et ses collaborateurs (1991, 1997) ont montré que les rats stressés suspendus en période prénatale présentent un desepoir comportemental dans le test de la nage forcée (Porsolt, 1978), un test classiquement utilisé pour la validation de l'efficacité des antidépresseurs.

De façon plus importante, dans ce modèle, les altérations reportées sont stables durant toute la vie, elles sont observées aussi bien à des stages précoces (Henry et al., 1994) qu'à des stages tardifs du développement (Vallee et al., 1999). La longueur du temps pendant lequel les effets persistent fait que le rat SP est un modèle avantageux pour les stratégies d'intervention.

2. Stratégies d'intervention dans le traitement des troubles dépressifs

Approche pharmacologique

Les buts du développement d'antidépresseurs efficaces sont d'avoir une action rapide et d'augmenter leur efficacité clinique tout en diminuant les effets indésirables. Les études neurobiologiques et neuroanatomiques indiquent l'importance des changements au niveau des systèmes noradrénergiques et sérotoninergiques pour un traitement antidépresseur efficace (Van Praag et al., 1990 ; Delgado et al., 1993 ; Cummings, 1993). Tous les antidépresseurs disponibles sur le marché agissent principalement sur la neurotransmission synaptique, soit en bloquant la recapture des monoamines, soit en inhibant la dégradation des neurotransmetteurs ou en les liant à des récepteurs spécifiques. Les antidépresseurs tricycliques (ATCs) et les inhibiteurs sélectifs de la recapture de la sérotonine (ISRS) sont les antidépresseurs les plus utilisés.

Les ATCs (imipramine, amytripiline) ont été introduits il y a environ quarante ans et sont encore aujourd'hui un traitement efficace stetard de la dépression. Le premier fut l'imipramine, puis l'amitripline qui est devenue un des antidépresseurs les plus utilisés, et enfin la clomipramine. L'imipramine et les autres ATCs inhibent la capture de la sérotonine et de la noradrénaline (NA). L'imipramine est un puissant inhibiteur de la recapture de la sérotonine alors que la désipramine, le principal métabolite de l'imipramine, inhibe la

recapture de la NA (Carlsonn et al., 1970). Tous les ATCs sont de faibles bloqueurs de la capture de la dopamine (Ross et Renyl, 1967). L'inconvénient majeur de l'utilisation des ATCs est du à leur large spectre d'action sur d'autres systèmes de neurotransmetteurs (cholinergique, histaminergique) et entraînant donc un gret nombre d'effets cliniques. Cependant, ces effets disparaissent habituellement au cours du traitement.

D'un autre côté, les ISRS, comme la fluoxétine, le citalopram et la paroxetine, augmentent la biodisponibilité de la sérotonine dans la fente synaptique, incluant les terminaisons et les corps cellulaires des neurones dans toutes les régions cérébrales. L'efficacité de cette classe d'antidépresseurs, spécialement chez les patients sévèrement dépressifs, n'est pas meilleure que celle des ATCs (Eterson et Tomenson, 1994). Cependant, la surdose est un problème moindre qu'avec les ATCs, car les ISRS produisent de multiples effets centraux et périphériques importants sur le plan clinique, mais minoritaires (Baldessarini, 1989).

La tianeptine est un nouvel antidépresseur tricyclique. Son activité thérapeutique est bien connue (Wagstaff et al., 2001), bien qu'elle ne présente pas l'action classique des antidépresseurs sur l'activité centrale des monoamines. Les études *in vivo* ont montré que, contrairement à la plupart des antidépresseurs, la tianeptine augmente la capture de la sérotonine De façon similaire aux ISRS et en opposition avec les ATCs classiques, la tianeptine entraîne peu d'effets indésirables ainsi qu'une faible propension aux abus. Les effets anticholinergiques se produisent moins souvent avec la tianeptine qu'avec les ATCs. De plus, il y a de plus en plus d'évidences que cet antidépresseur peut prévenir, voire même inverser les changements induits par le stress dans la morphologie cérébrale, souvent associés à des troubles psychiatriques liés au stress (McEwen et al., 1997; Margarinos et al., 1999;Czeh et al., 2001).

Deux problèmes majeurs apparaissent dans le traitement de la dépression par les antidépresseurs. Le premier est que, quel que soit le type d'antidépresseur utilisé, les bénéfices des drogues sont visibles seulement après plusieurs semaines d'administration continue (Dubovsky, 1994; Blier et de Montigny, 1994; Nestler, 1998). Le second est qu'il y a une grande incidence dans la rechute et dans la récurrence des épisodes dépressifs après interruption du traitement. Ceci suggère que les antidépresseurs sont des drogues agissant sur les symptômes et n'entraînant pas de façon systématique une thérapie efficace. De plus, parmi les patients dépressifs, on peut noter une grande variabilité dans la sensibilité au traitement aux antidépresseurs, en fait il y a plus d'un tiers des patients qui ne répondent pas au traitement (Quitkin et al., 1996; Joyce et Paykel, 1989).

Approche environnementale

Chez les patients dépressifs, le traitement de la dépression majeure est souvent multimodal et utilise habituellement la pharmacothérapie et la psychothérapie (Burns et al., 2002). Il y a également des données sur une stimulation positive de l'environnement qui peut moduler l'occurrence et/ou la rechute du trouble dépressif. Par exemple, la stimulation environnementale que forme un support social exerce un rôle protecteur important, car un support social pauvre est lié à la rechute des épisodes dépressifs (Brugha, 1990). Ainsi, une aide avec des techniques sociales comme l'intégration à des équipes de sport ou à des groupes d'intérêt pourrait améliorer l'issue de la maladie.

Il a été montré que les enfants avec un degré élevé d'intelligence, une bonne résolution des problèmes, un bon support social et une haute estime de soi sont moins amenés à devenir dépressifs que les autres lorsqu'ils sont confrontés à des risques environnementaux. Ajoutée aux caractéristiques individuelles, la présence du support social joue un rôle protecteur important (Seifer et al., 1992). Les enfants qui grandissent au sein de situations familiales négatives seront moins amenés à être dépressifs s'ils ont une relation de confiance avec au moins un adulte en dehors de leur famille ou s'ils sont impliqués dans des activités communautaires et obtiennent une reconnaissance scolaire positive (Cheung, 1995).

La manipulation de l'environnement par augmentation de sa complexité (environnement enrichi) est depuis longtemps employé dans des études sur le comportement et le cerveau comme un moyen d'étudier les mécanismes biologiques sous-tendant le comportement et pour modéliser les symptômes des troubles psychiatriques de l'humain (voir Diamond, 2001). Chez l'homme, l'enrichissement d'environnement post-traumatique ou préopératoire a été utilisé comme accessoire dans la récupération de blessures cérébrales variées (Will et Lelche, 1992).

Chez l'animal, il a été montré que l'entraînement à des tâches spatiales complexes et le fait d'être dans un environnement complexe modifie la neurochimie et le poids du cerveau chez le rat (pour revue, voir Renner et Rosenzweigh, 1987). L'environnement enrichi change les taux du facteur de croissance neuronal (Mohammed et al., 1990), altère l'activité de l'axe HHS chez les jeunes (Francis et al., 2002) comme chez les animaux âgés (Mohammed et al., 1993) et affecte les profils de sommeil (Mirùiran, 1982). Cette procédure expérimentale a des effets bénéfiques sur les maladies neurodégénératives (Van Dellen et al., 2000) et sur le vieillissement (Kempermann et al., 1998) et il y a de plus en plus des données montrant une neurogénèse en réponse à l'enrichissement chez un nombre d'espèces variées.

Parmi ces multiples effets, l'environnement enrichi semble partager des substrats d'action avec le traitement aux antidépresseurs (Blier et de Montigny, 1999) et il pourrait constituer une approche thérapeutique alternative et intéressante.

Une partie de ce travail de thèse, le chapitre 2, est consacrée à l'évaluation de l'impact des deux stratégies d'intervention différentes, le traitement chronique aux antidépresseurs et l'environnement enrichi, dans la réversion des anormalités induites par le stress prénatal.

OBJECTIFS SPECIFIQUES DE LA THESE

Chapitre 1/ Cette partie de mon travail de thèse pose le problème de l'influence de l'environnement prénatal sur la vulnérabilité à l'abus de drogues lors de l'adolescence, en utilisant deux modèles d'expérience de vie précoce : la position intra-utérine (PIU) chez la souris et le stress prénatal (SP) chez le rat.

Dans la première partie notre but était d'évaluer l'impact de la PIU sur un comportement dimorphique au niveau sexuel, tel que le comportement de recherche de nouveauté, lors de l'adolescence chez la souris. Comme ce comportement prédit une sensibilté différente à l'abus de drogues, les animaux ont été observé sur le plan neurochimique et comportemental à l'âge adulte et en réponse à l'administration d'opiacées. Les résultats obtenus dans ces expériences indiquent que l'exposition à des niveaux croissants de testostérone en conséquence de la proximité avec des fœtus mâles lors de la période prénatale peut augmenter le comportement de recherche de nouveauté et, ainsi, la sensibilté aux psychostimulants. Cette recherche a permis la publication de deux articles.

- Palanza, P., Morley-Fletcher S., Laviola G. (2001). Novelty seeking in periadolescent mice: sex differences et influence of intrauterine position. Physiology & Behavior 72: 255-262.
- Morley-Fletcher S., Palanza P., Parolaro D., Vigano D., Laviola G. (2002). Intrauterine position has long-term effects on mu opioid receptor density et behaviour in mice. Psychoneuroendocrinology (sous presse).

Dans la seconde partie, le but était d'évaluer la vulnérabilité des rats SP à une drogue récréative tel que l'ecstasys lors de la période de développement de l'adolescence. Les effets de l'ecstasy ont été évalués lors d'une tâche psychomotrice, et les propriétés pharmacocinétiques de la drogue dans le sang ont également été étudiées. Les résultats obtenus dans ce travail confirment l'augmentation de la vulnérabilité aux psychostimulants chez les animaux SP et étendent ces données aux rats adolescents qui ont été décrits chez l'adulte. Cette recherche a abouti à l'élaboration d'un article en cours de préparation.

- Morley-Fletcher S., Puopolo M., Gerra G., Gentili S., Macchia T., Laviola G. Prenatal stress affects pharmacokinetics et behaviour following MDMA ("ecstasy") administration during adolescence. European Journal of Pharmacology (soumis)

Chapitre 2/ La seconde partie de mon travail de thèse évalue le rat SP comme un modèle animal de psychopathologie et questionne la possibilité de reverser ces altérations par deux approches : une approche pharmacologique lors de l'âge adulte et une approche environnementale lors de l'adolescence. Les résultats de cette partie ont été présentés dans le chapitre 3.

Dans la première partie, nous avons évalué la "predictive validity" du rat SP comme modèle animal de dépression en utilisant un traitement chronique avec deux antidépresseurs différents tels que la tianeptine et l'imipramine. Les animaux SP ont été caractérisés dans différents tests comportementaux et à l'aide différents analyses neurochimiques. Les résultats obtenus indiquent que les rats SP répondent positivement au traitement antidépresseur au niveau comportemental et neurochimique, renforçant ainsi la "predictive validity » de ce modèle. Cette recherche a abouti à l'écriture de deux articles.

- Morley-Fletcher S., Darnaudery M., Koehl M., Munoz C., Casolini P., Van Reeth O. Maccari S. High corticosterone levels in prenatally stressed rats predict immobility behaviour in the forced swim test. Effects of a chronic treatment with tianeptine. Brain Research (soumis).
- Morley-Fletcher S., Darnaudery M., Mocaer E., Froger N., Lanfumey L., Laviola G., Casolini P., Zuena A., Hamon M., Maccari S. Chronic treatment with imipramine affects behaviour, hippocampal corticosteroids et cortical 5-HT1A receptor expression in prenatally stressed rats (en préparation).

Dans la seconde partie, nous avons utilisé un environnement enrichi lors de la période de développement de l'adolescence pour reverser les altérations induites par le SP. Les résultats obtenus indiquent que les rats SP élevés dans un environnement enrichi présentent une réduction des anormalités induites par le SP au niveau comportemental et au niveau de la réponse au stress. Cette recherche a abouti à l'écriture d'un article soumis.

- Morley-Fletcher S., Rea M., Maccari S., Laviola G. Environmental enrichment during adolescence reverses the effects of prenatal stress on anxiety-related behaviours et stress reactivity in rats. European Journal of Neuroscience (soumis).

RESULTATS

RESULTATS

CHAPITRE 1

NOVELTY SEEKING IN PERIADOLESCENT MICE: SEX DIFFERENCES ET INFLUENCE OF INTRAUTERINE POSITION.

Palanza, P., Morley-Fletcher S., Laviola G.

Physiology & Behavior (2001) 72: 255-262.

INTRAUTERINE POSITION HAS LONG-TERM EFFECTS ON MU OPIOID RECEPTOR DENSITY ET BEHAVIOUR IN MICE.

Morley-Fletcher S., Palanza P., Parolaro D., Vigano D., Laviola G.

Psychoneuroendocrinology , sous presse.

La PIU détermine les niveaux hormonaux fœtaux car les stéroïdes sexuelles endogènes sont transportés d'un fœtus à l'autre, modulant ainsi l'action organisatrice des hormones sexuelles.. Ajouté aux périodes critiques pré-natales et néonatales bien connues, durant lesquelles le cerveau est organisé par les stéroïdes sexuelles, la puberté représente également une phase développementale importante.. Lors de cette période, et en parallèle avec le développement des fonctions reproductrices, les individus acquièrent différentes habiletés qui leur permettent de devenir indépendants.. Nous avons conduit une première étude, dont le but était d'évaluer les interactions entre les effets à long terme de la PIU et les effets activateurs des hormones sexuelles circulantes lors de l'adolescence, sur l'expression d'un comportement sexuellement dimorphique tel que l'exploration (Beatty et al., 1979) chez les souris des deux sexes et de PIU connues. Les rongeurs adolescents montrent des niveaux élevés d'activité locomotrice de base et d'activité exploratoire comparé aux adultes et sont caractérisés par une augmentation de la sensibilité aux psychostimulants (Adriani et al., 1998).

En prenant en compte ces considérations, nous avons émis l'hypothèse que l'influence de la PIU sur le comportement de recherche de nouveauté serait liée à son influence sur la réponse de l'animal aux psychostimulants.

- Les mâles ont montré une recherche de nouveauté supérieure aux femelles et les mâles entourés de deux mâles in utero (2M) exprimaient un profil de recherche de nouveauté plus élevé que les mâles entourés de deux femelles dans l'utérus (0M). La position utérine n'affecte pas le profil de recherche de nouveauté chez les femelles.
- L'autoradiographie a révélé une densité des récepteurs opioïdes mu augmentée dans le cerveau médian, chez les mâles et dans le striatum, chez les femelles.

- Les femelles 1M (entre un mâle et une femelle) et 2M ont montré des niveaux de densité plus élevés dans le cerveau médian que les sujets 0M, alors que les mâles 1M et 0M ont montré une densité de récepteurs augmentée au niveau du striatum comparé au groupe 2M.
- Le test de préférence de place conditionnée par la drogue a montré que les sujets 1M et 2M sont plus sensible aux effets de récompense de la drogue, car ces souris passent plus de temps dans le compartiment associé à la drogue que les sujets 0M. Dans le test de la plaque chauffante, les sujets 2M ont montré des niveaux plus élevés d'analgésie induite par la drogue que les sujets ayant d'autres PIU.

Ces résultats indiquent que la position utérine a des effets à long terme sur les capacités adaptatives d'un individu et que des variations subtiles dans l'exposition prénatale à la testostérone peuvent affectent la sensibilité aux psychostimulants.

PRENATAL STRESS IN RATS AFFECTS PHARMACOKINETICS ET BEHAVIOUR FOLLOWING MDMA ("ECSTASY") ADMINISTRATION DURING ADOLESCENCE

Morley-Fletcher., Puopolo M., Gerra, G., Gentili S., Macchia T., Laviola G.

(soumis à European Journal of Pharmacology)

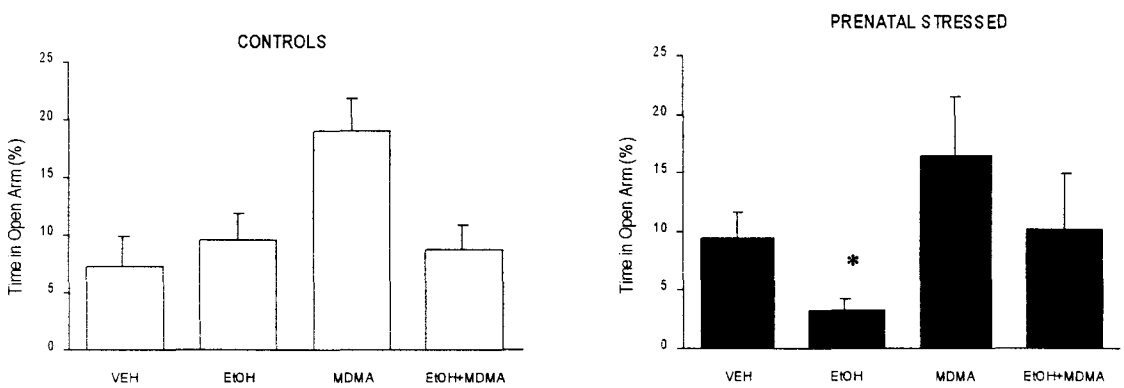
Dans la troisième étude, nous avons examiné les effets du SP sur la vulnérabilité au MDMA lors de l'adolescence chez des rats femelles (âgées de 30 jours). Le MDMA est une drogue à la popularité grandissante chez les jeunes en raison de ses effets euphorisants, de sentiments d'intimité et d'empathie (Cami et al., 2000). Cependant, plusieurs troubles comportementaux et neurochimiques sont associés à la consommation de MDMA et vont des troubles cognitifs jusqu'à la neurotoxicité et des défaillances dans la coordination motrice (McNamara et al., 1995; Daws et al., 2000; Maldonado et Navarro, 2001; Schifano et al., 1998).

- Les animaux SP présentent une augmentation du nombre des altérations motrices dues à la drogue telles qu'une augmentation de l'activité et du déséquilibre dans un test psychomoteur.
- Les rats SP présentent des valeurs élevées de MDMA circulant durant toute la période d'évaluation en comparaison avec d'autres animaux. De plus, une corrélation positive a été trouvée entre les niveaux de MDMA et le profil des altérations motrices représentées par le nombre de retournements réalisés par les animaux dans le test de coordination motrice

Effets du stress prénatal sur la polytoxicomanie à l'adolescence (étude en cours dans le laboratoire de Stress Périnatal)

Les adolescents consommateurs de drogues sont typiquement des poly toxicomanes et cela pourrait contribuer aux effets opposés observés (Spear, 2000). Nous avons conduit une étude visant à examiner la vulnérabilité individuelle à la consommation à la fois d'alcool et de MDMA chez les adolescents mâles aussi bien que sur l'influence du SP sur ce phénomène. Chez l'homme (Bates et Laboucie, 1997) et chez les rongeurs (Silveri et Spear, 2000), les adolescents sont relativement insensibles aux altérations motrices et aux effets sédatifs de l'alcool, cela pourrait leur permettre de supporter de plus grande quantité d'éthanol en comparaison aux adultes.

Les rats mâles adolescents (âgés de 30 jours) ont reçu un traitement aigu avec du MDMA (5 mg/kg) ou avec de l'éthanol (EtOH, 1.2 mg/kg) ou EtOH + MDMA et sont ensuite examinés dans le labyrinthe en croix surélevé pour évaluer les altérations de leur comportement émotionnel. Un traitement aigu à l'éthanol induit des effets anxiogènes chez les rats SP ($P>0.05$ vs VEH), alors que le MDMA exerce une action anxiolytique chez les groupes SP et contrôle. Le mélange EtOH+MDMA n'a pas d'effet. Les animaux SP apparaissent plus vulnérables aux effets de l'EtOH que les contrôles.



Ces résultats indiquent que le SP induit, au stade précoce du développement lors de l'adolescence, une sensibilté plus élevée à une grande variété de psychostimulants (nicotine, amphétamine, MDMA, alcool). Cette réponse augmentée pourrait être due, en partie, aux différences précoces du métabolisme des psychostimulants.

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PHYSIOLOGY & BEHAVIOR

Novelty seeking in periadolescent mice: sex differences and influence of intrauterine position

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Abstract

In rodents, beside basic sex differences, a certain degree of within-gender phenotypic variation can also be provided in utero by hormones from adjacent fetuses. We investigated novelty-seeking behavior in two groups of male and female mice from know intrauterine position: 2M (between males) and 0M (between females). Subjects were assessed during periadolescence (postnatal days 33–43), an ontogenetic phase, which is characterized by an elevated expression of this novelty-seeking behavior. Periadolescent mice underwent a familiarization session for 3 consecutive training days with one side of a two-chamber apparatus. On testing day 4, the opening of a partition, which allowed mice to freely move from the familiar compartment to a novel one, produced an increased behavioral arousal in all animals. Marked sex differences were found, with females being in general more active than males, whereas the latter showed significantly higher levels of novelty seeking than females. Uterine position failed to affect the profile of novelty preference in females, whereas within the male group 2M subjects expressed a marked profile of novelty seeking. The differential titers of sex hormones reported to characterize the 0M and 2M condition early in fetal development are suggested to account for the individual variability in the seeking for novelty within the male group during puberty. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Intrauterine position; Novelty seeking; Gonadal hormones; Mice

In rodents, many nonreproductive behaviors have been described to show sex differences, which do not necessarily imply the presence of a given response in one sex and its absence in the other, but rather differences in quantity of performance expressed [24]. In all multiparous rodent species examined to date (e.g., mice, rats, and gerbils), in addition to endogenous sex hormones, which are known to be responsible for those differences, a certain degree of variability within the sexes has been found to be accounted by exposure during fetal life to hormones of siblings [14,29,53,55,56] (for relevance in humans, see Refs. [28,41]). In fact, the position of male and female fetuses within the uterus, in relation to the sex of the adjacent litter mates, would affect the transmission of the excreted gonadal hormones by one fetus to contiguous fetuses, thus modulating the internal hormonal milieu of coresident of a uterine horn during a prenatal sensitive period. Male and female fetuses are known to secrete different titers of steroid hormones during the prenatal period of sexual differentiation. Radioimmunological studies conducted by vom Saal et al. [43,54,57] showed that fetuses location between adjacent littermates of the opposite sex enhanced blood titers of testosterone for 2M females (gestated between two males), and titers of estradiol for 0M males (gestated between two females). A study by Baum et al. [6] did not confirm these effects. However, methodological procedures were markedly different.

For both sexes, uterine position has been involved in many parameters, among which are rate of bodyweight gain, sexual attractiveness, parental care, aggressiveness, activity patterns, and avoidance responding when adult [27,34,51,56]. Differences between 2M and 0M females have been described for anogenital distance (AGD) in some specific mouse strains and for estrous cycle length [23,54,57]. However, it should be noted that, at least for females, negative results on intrauterine positions (IUP) phenomenon have also been reported. Thus, the analysis of IUP phenomenon seems to deserve further investigation in

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additional behavioral paradigms [15,22,23], as well as in different mammalian species and stages of development.

Individual differences in the way to meet novel stimuli, like in any other trait, may be caused by genetic and/or environmental differences, and their interactions. To date, a number of studies have addressed the role of sex steroids exposure during perinatal life in influencing responses to changes in the environment, and more generally behavioral strategies in coping [3,7–9]. In this view, the endocrine environment surrounding a fetus could exert a contribution in modulating this profile.

Animals are biologically designed to pay more attention to novel information than to a familiar one, and they actually seem to be both attracted and activated by novel stimuli, as well as by variations in the set or the intensity of familiar ones [32,36]. The response to a novel environment in a free-choice paradigm has been proposed as the way of studying experimentally this parameter and individual differences in reactivity to novelty have been described [18,37,49] (for human studies, see Refs. [46,63]). Experience of novelty (i.e., entering a novel environment of an apparatus) in rats is associated with increased behavioral arousal and the activation of reward-related brain areas. The first aim of the present mouse study was to analyze sex differences in reactivity to a free-choice novelty paradigm.

Animals were tested during periadolescence, which has been defined as the ontogenetic period that encompasses the 7–10 days preceding the onset of puberty (at about 40 days of age in rats and mice at least for males) and the first few days thereafter [48]. Periadolescent rats and mice are characterized by elevated basal levels of behavioral activation and a high propensity for the expression of an affiliative and playful behavioral repertoire [13,42,50]. We recently showed [1] that when compared to adult subjects, periadolescent mice particularly expressed elevated levels of novelty seeking. Furthermore, there is increasing evidence in the clinical literature concerning the association of high levels of sensation novelty seeking with the expression of risky behaviors and individual vulnerability to drugs of abuse during adolescence [4,16,37]. This ontogenetic period is also characterized by a prominent increment of gonadal hormones, with the consequent sexual maturation of each individual's physiological and behavioral patterns [44]. The second aim of the work described here was therefore to assess a possible influence of variation in the hormonal milieu related to intrauterine position on the natural willingness to search for novel stimuli and coping response to changes in the environment.

1. Materials and methods

1.1. Subjects

Animals of the outbred CD-1 mouse strain, without prior breeding experience, were purchased from a commercial breeder (Charles River Italia). On arrival, mice were housed in an air-conditioned room (temperature, 21 ± 1°C; relative humidity, 60 ± 10%) with a reversed 12-h light–dark cycle (lights on at 8:00 PM). Water and food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, Italy) were available ad libitum. To obtain offspring from known intrauterine position, nulliparous females were time mated by placing them with a sexually experienced male. Upon discovery of a vaginal plug (designated as gestation day 0), the females were housed in pairs in Plexiglas cages (33 × 13 × 14 cm) and after approximately 2 weeks, housed individually.

The following procedure has been used adopting the method described by vom Saal and Bronson [54]. On day 18 of gestation (generally 8 h prior to term), each female was sacrificed by cervical dislocation, a midline incision was made down the ventrum, and the uterine horns were exposed. Each fetus was immediately removed in the order found, gently cleaned with water, sexed, weighed, and its intrauterine position recorded. The wet fetuses were next placed under heat lamps to keep warm. AGD was measured for all the pups using a Zeiss (Germany) microscope with a micrometer lens (accurate to 0.05 mm). One person to whom pups were passed made measurements without knowledge of intrauterine position. Pups were unequivocally marked using a toe-clipping pattern to allow identification.

The following two groups of animals were formed: animals from each sex that resided between two females (0M: $n = 12$ females and 24 males), and between two males (2M: $n = 26$ females and 24 males). Subjects located next to one male fetus and one female fetus (1M) were not used in this experiment having being reported to show behavioral and morphological characteristics intermediate between that of 0M and 2M subjects [56]. Within no more than 10 min from delivery, the neonates were next fostered to lactating female mice who had delivered vaginally during the prior 24–48 h. Litters were culled to eight sibling pups with four males and four females when possible. Mouse pups were weaned on day 21 and housed in each Plexiglas cage (33 × 13 × 14 cm), according to sex in groups of four to six. Body weight gain was followed on days 7, 14, 21, and 36 after birth.

A great effort was devoted to minimize possible cannibalism occurrence following fostering. Specifically, the dam to become a foster mother was temporarily moved to a clean cage. Her pups were carefully removed without altering the nest structure and the cesarean-delivered pups were placed into the nest and covered with the nest material. Pups were warm and moving when introduced into the nest and the toe-clipped mark was completely recovered.

1.2. Apparatus

The experimental apparatus for the Novelty Preference paradigm consisted of an opaque Plexiglas rectangular box with smooth walls, subdivided into two compartments (20 × 14 × 27 cm). The connection door between the two

compartments could be closed by means of a temporary partition. Mixed visual cues were associated with both compartments. One compartment had white walls and a black floor, whereas the other one had black walls and a white floor. Each compartment was provided with four infrared photobeams, placed on the wall at few centimeters from the floor. Each beam interruption eventually caused by mice was recorded by an IBM computer. The floor and the wall of the apparatus were washed with a solution of water and ethyl alcohol (2%) after each animal was trained or tested.

1.3. General procedure

Mice were assigned for testing at periadolescence (postnatal days 33–43), following the procedure described in previous experiments (for more details, see Ref. [1]). The whole experimental schedule took a total of 4 days, each subject being trained and tested between 10:00 AM and 6:00 PM. Testing of different sex and intrauterine position groups were counterbalanced across time. Training and testing were carried out under dim illumination.

1.3.1. Days 1, 2, and 3: familiarizarion

Animals were weighed and immediately placed for 20 min in one compartment of the apparatus, namely, the Familiar compartment.

1.3.2. Day 4: novelty preference test

Animals were placed in the Familiar compartment. After a 5-min session, the partition separating the two compartments of the apparatus was removed, and mice were thus allowed to freely explore both compartments of the apparatus (the Familiar and the Novel ones) for 20 min.

The following measures were obtained automatically: (1) time spent in each compartment; (2) locomotor activity in each compartment (number of beam interruptions/s). The whole session was automatically subdivided into 5-min intervals.

1.4. Design and statistical analysis

Data were analyzed by using parametric analysis of variance (ANOVA) with two levels of sex and two levels of IUP as between subjects factors. Repeated measures were considered as within subject factors [12,59]. With respect to AGD, data were analyzed by both ANOVA and analysis of covariance (ANCOVA), with body weight used as the covariate, to determine whether some portion of the variance in AGD might be accounted for by differences in body weight [60]. Multiple comparisons within a significant interaction were performed using the Tukey HSD Test.

2. Results

2.1. Anogenital distance

Data refer to all pups delivered by cesarean section before culling. Statistical analysis was conducted by ANOVA, which revealed a main effect of sex on AGD, $F(1,158)=98.30$, $P<.01$, and weight $F(1,158)=8.74$, $P<.005$, with males presenting higher value than females for both parameters. In relation to females' IUP, 2M group presented slightly longer AGD than 0M animals (1.49 ± 0.05 vs. 1.43 ± 0.04 mm). Further, AGD data analyzed by ANCOVA using body weight as the covariate revealed that body weight did not accounted for the variance in AGD.

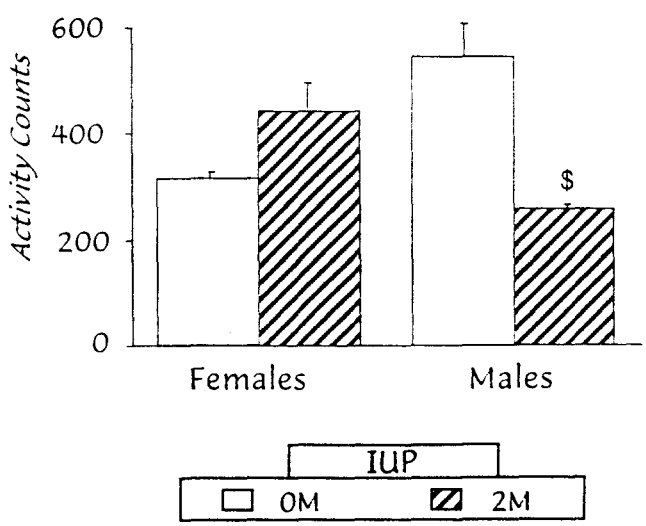

Fig. 1. Mean (±S.E.M.) locomotor activity, measured automatically as number of photobeam interruptions/s during a 20-min session/day, shown by periadolescent mice of both sexes and uterine positions on familiarization days 1, 2, and 3 (see Materials and methods). Data are shown as a pool of these days. $^{\$}P<.05$ in multiple comparisons performed between 2M males vs. 0M males ($N=12$ females and 24 males in the 0M group; 24 females and 26 males in the 2M group).

Table 1
Mean number (±S.E.M.) of transitions between the familiar and the novel compartment shown by subjects of both sexes and IUP on test day 4 after the opening of the partition (single 20-min session)

IUP	0M females	0M males	2M females	2M males
	15.95±1.07	14.71±0.60	19.88±1.18**	12.23±0.71

N=12 females and 24 males in the 0M group; 24 females and 26 males in the 2M group.
** $P<.01$ in multiple comparisons between sexes within the 2M group.

2.2. Body weight gain

Animals were followed for body weight gain and the ANOVA yielded a main effect of sex, $F(1,79)=28.18$, $P<.001$, as well as an interaction with days, $F(4,316)=41.75$, $P<.001$. As expected, males were in general heavier than females with time. In addition, an IUP × Day interaction was found, $F(4,316)=3.39$, $P<.01$, with 0M subjects weighing lightly but consistently more than the 2M group. Separate analyses performed for the two sexes, revealed that IUP related differences were particularly evident within the male group [IUP × Day interaction, $F(4,172)=4.73$, $P<.001$; particularly on day 36: 0M male subjects 29.34 (±0.513) g vs. 2M mice 27.22 (±0.69) g].

3. Days 1, 2, and 3 (familiarization period)

3.1. Activity

For locomotor activity data collected over the 3 days of training, a significant Sex × IUP interaction appeared, $F(1,81)=4.81$, $P<.05$. As shown in Fig. 1, multiple comparisons revealed that in the absence of significant differences within the female group, 0M males performance was significantly higher than the corresponding 2M group ($P<.05$).

4. Day 4 (testing day)

4.1. Transitions

On the day of testing, after the partition was opened, mice showed a number of transitions between the familiar and the novel compartment. An ANOVA carried out on these data revealed a main effect of sex, $F(1,80)=8.84$, $P<.01$, with females as a whole showing as expected significantly higher levels than males (see Table 1). In addition, a Sex × IUP interaction, $F(1,80)=4.60$, $P<.05$, was found with 2M females performance resulting significantly higher than the corresponding male group ($P<.01$). No significant difference between 2M and 0M females was revealed.

4.2. Novelty seeking

With respect to the amount of time spent in the Novel compartment, the ANOVA yielded significance for a Sex × Repeated measures interaction, $F(3,240)=3.49$, $P<.01$

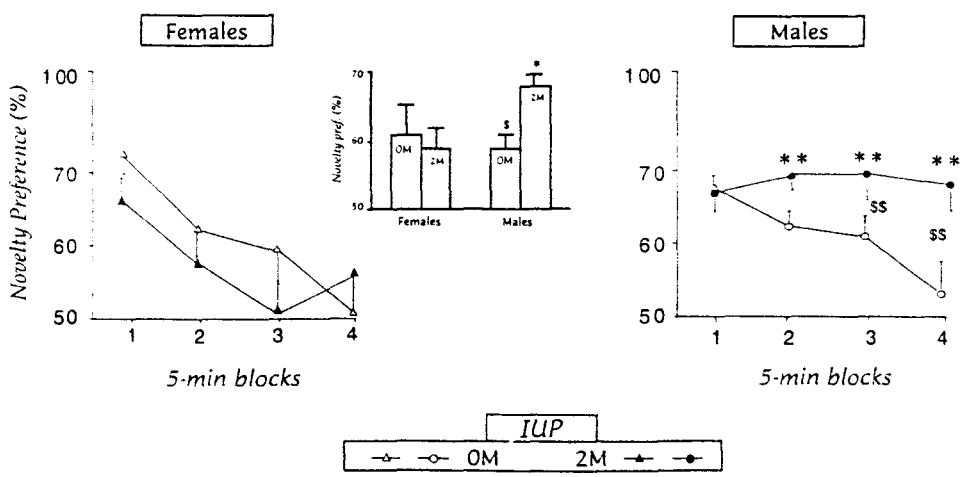

Fig. 2. Mean (±S.E.M.) percentage of time spent in the novel compartment Novelty seeking) presented as a function of repeated measures during the 20-min session (four 5-min intervals) after the partition opening. $^{SS}P<.01$ in multiple comparisons performed between 2M males vs. 0M males; $**P<.01$ in multiple comparisons performed between 2M males vs. 2M females (N=12 females and 24 males in the 0M group; 24 females and 26 males in the 2M group). Inset: the same data of this figure are presented pooled over the session on test day 4 by periadolescent mice of both sexes and uterine positions. $^{S}P<.05$ in multiple comparisons performed between 2M males vs. 0M males; $*P<.05$ in multiple comparisons performed between 2M males vs. 2M females (N=12 females and 24 males in the 0M group; 24 females and 26 males in the 2M group).

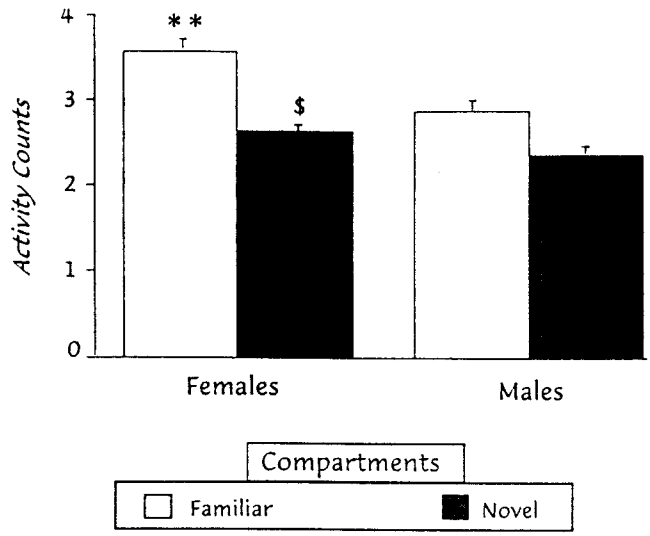

Fig. 3. Mean (±S.E.M.) activity rate (number of photobeam interruptions/time spent in the compartment) in both familiar and novel compartments, shown by subjects of both sexes and uterine positions on test day 4, after the partition opening (single 20-min session). Data are shown pooled over the IUP variable. $^{\$}P<.01$ in multiple comparisons performed within the males or females group respectively. $^{**}P<.01$ in multiple comparisons performed between sexes within the familiar or novel groups, respectively (N=38 females and 48 males).

(see Fig. 2), with males showing as a whole consistently higher levels of novelty seeking, as well as a less marked habituation profile over the session than females. In addition, an IUP × Repeated measures significant interaction was found, $F(3,240)=5.16$, $P<.01$. 0M males spent reduced time in the novel compartment and habituated faster than the 2M group.

A significant Sex × IUP interaction, $F(1,80)=4.73$, $P<.05$, also appeared (see inset Fig. 2), with 2M males spending as a whole the highest portion of time in the novel compartment. The performance of 2M male mice (see Fig. 2 right panel) was characterized by a constant and elevated profile throughout the testing session, and difference in comparison with the corresponding 0M males and the 2M female group reached significance from the middle of the session onward ($P<.01$).

4.3. Activity

The final test consisted of two phases, i.e., before and after the partition opening. A preliminary ANOVA, considering data from the 5 min before and the first 5 min after the partition opening, revealed a main effect of phase, $F(1,80)=408.44$, $P<.001$, mouse activity being as a whole significantly higher after that the partition was opened than before (number of photobeam interruptions 1.5±0.11 before, 3.5±0.11 after). Furthermore, an analysis considering the different potential influence of the familiar vs. the novel environment on the mouse performance after the partition opening was carried out on data of activity rate. As shown in Fig. 3, a significant main effect of side was found, $F(1,78)=84.26$, $P<.01$, with reduced levels of activity being exhibited as a whole when mice were spending time in the novel compartment. In addition, a Sex × Side interaction, $F(1,78)=7.13$, $P<.01$, and multiple comparisons indicated that a prominent sex difference appeared, with females exhibiting in general higher values than males in the familiar compartment. Interestingly, whereas no significant changes were found in the male group, females exhibited a significant reduction in activity rate when spending time in the novel compartment ($P<.01$). In general, no carry over effects of IUP variable were found.

5. Discussion

The main findings of the present study can be summarized as follows.

(1) For levels of novelty seeking, a marked sex difference was evident with periadolescent males spending in general a significantly higher percentage of time in the novel compartment when compared to females.

(2) For the long-term influence of IUP, a prominent novelty-seeking profile, as well as a reduction of activity levels, was particularly associated with 2M periadolescent males.

(3) As expected, a sex difference appeared for activity levels, with females exhibiting much higher values than males. This profile appeared associated with the familiar compartment, whereas it was significantly reduced when females were spending time in the novel one.

Periadolescent animals of both sexes expressed a clear-cut preference for novelty. This finding is in agreement with a previous report [1], which compared novelty seeking in adult and periadolescent mice; the latter spent more time in

the novel compartment than adult subjects. Elevated levels of novelty seeking appear to be highly adaptive for young animals, as it is during the periadolescent period that rodents are seen to begin to explore at some distance from the nest site and later start to disperse [19,21].

The discovery of a novel environment and the possibility to free access to it, also elicited a prominent increment of general activation, clearly indicating that the experience of novelty had an arousing effect in animals of both sexes. In fact, levels of locomotion were as a whole much higher after the partition opening than before. An interesting finding that confirms and extends previous observations [1,5,36] is that when mice were involved in the exploration of the novel compartment, their levels of locomotor activity were always and consistently lower than those expressed in the familiar one. A similar profile has been previously interpreted as a byside effect of the assessment of potential danger in a completely unknown environment (for literature and discussion, see Ref. [36]). Thus, we showed evidence that two kinds of phenomena were elicited by the experience of novelty, namely an increased general arousal and a slight behavioral inhibition.

In agreement with previous reports [7,10], we found female mice to be in general more active than males and this sex difference was particularly marked in the familiar environment. Conversely, when subjects were spending time in the novel compartment, the sex difference in locomotor activity was strongly reduced with females reaching lower male-like levels. This female-associated behavioral pattern confirms previous reports of a differential reactivity to changes in environment found in the two sexes [25,45,47]. In this line, a clear-cut sex difference also appeared for the novelty-seeking profile, with males showing significantly higher novelty preference when compared to females. An ecoethological explanation could consider that in most mammalian species natal dispersal is sexually dimorphic, with males that reach puberty usually emigrating from their natal areas [11,19], whereas females are typically philopatric [17].

With respect to the long-term influence of intrauterine position, a significant difference was evidenced during the training familiarization period, with 0M males being associated with higher levels of activity than 2M subjects. Similar results were also obtained after the opening of the partition during the test session. Again, periadolescent 0M males expressed higher activity levels than 2M male subjects. A few comments should be paid on some aspects of these results.

As reported by vom Saal [55,57], 0M and 2M males are suggested to differ in levels of testosterone and estradiol, and both systems may account for observed differences. It has been suggested [34,53] that at least for some behaviors, whose expression is thought to be organized during early life, estradiol may synergize with testosterone (i.e., sexual performance). In other instances such as aggression or locomotor activity, the former steroid may compete with androgens and act as an antihormone in some tissues [34]. In this view, as outlined by Fitch and Denenberg [20], gonadal hormones can act independently or interact with each other, and there is increasing evidence that estrogen (partially of ovarian origin) may play an active role in development of the brain [26]. In other system including development, early exposure to androgens may prime estrogen responses (e.g., via upregulation of aromatase) or estrogen may prime androgen responses (e.g., via receptor induction). It has been well known that estradiol plays a role in normal development of male sex behavior. However, it was thought that all of the estradiol present in the brain and other tissues came of conversion of testosterone by aromatase. This hypothesis was demonstrated to be incorrect in a series of studies [42,58], which have demonstrated that estrogens have a direct effect on androgens. Furthermore, recent reports [20] suggest that the various components of male vs. female differences in brain structures and functions may become expressed during different developmental periods and in response to different hormonal influences.

A similar mechanism could be taken into account when considering the significant differences evidenced within the male group for the novelty-seeking profile. In fact, in agreement with the reports of a within-sex variability, periadolescent 2M males spent the highest portion of time in the novel compartment when compared to other groups, and were also increasingly novelty seekers as the session progressed (see Fig. 2). Testosterone has been shown to be responsible for sex differences in this parameter, promoting dispersal behavior through its influence during perinatal life [30]. Again, a competitive interaction between testosterone and ovarian-derived estrogen as a consequence of contiguity to female fetuses could be hypothesized for the low novelty-seeking levels exhibited by 0M males.

An interesting finding that emerges from our study is that apparent inconsistencies in IUP effects probably reflect differences in the way slight changes in testosterone and estradiol levels interfere with basic regulatory mechanisms during development in the two sexes. In the present work, there was some tendency for within female variation accounted to be IUP that failed to reach significance. With respect to AGD, only a slight and non significant difference between 0M and 2M females was found, which is consistent with a previous report [33]. It should be noted that up to date positive IUP effects on this parameter have been evidenced only in very selected mouse strains [52]. With respect to activity patterns, again no significant or reliable changes related to the IUP variable were found in the female group. This profile is in apparent contrast with previous work, which interpreted a reduction in levels of locomotion in 2M adult females in light of the modulatory action of increased levels of testosterone in the uterine milieu [10,34]. It should be noted, however, that subjects of the present study were mice observed during periadolescence, an ontogenetic phase still characterized by important maturational rearrangements in brain/behavior regulations.

Thus, differences due to experimental procedure, genotype and maturational stage of the subjects should be considered (see also Ref. [61]).

It could be considered that the differential response to a social and environmental stimuli, which has been shown to characterize animals from different IUPs [55], may be a reflection of an acquired difference in coping styles. Early ontogeny is a markedly plastic and crucial stage [35,38], and alterations of hormonal milieu during fetal development may have a major role in modulating developmental trajectories that, in turn, will influence subsequent behavioral responses [62]. Present findings indicated for the first time that at least for male subjects, developing in a 2M or 0M condition can have important consequences on the way mice, around puberty, cope to changes in the environment. This may suggest differences in emotionality, with 2M males being consistently associated with slower habituation to the novel environment, suggesting a lower trait anxiety (see Ref. [39]). Conversely, 0M males showed a more reactive profile, which is suggestive of a higher anxiety trait. This hypothesis needs to be further investigated with appropriate indexes of emotionality, such as for example the defecation score [3].

For relevance of this kind of studies, there is increasing evidence in clinical literature concerning the association of high levels of sensation/novelty seeking with the expression of risky behaviors and individual vulnerability to drugs of abuse during adolescence [2,4,5,16,36,37,40]. Our data derived from an animal model, indicate that individuals exposed to subtle variations of sex hormones during prenatal life as function of uterine position, and with differing sensitivity to these hormones as a function of sex of subjects, may also exhibit a peculiar responsivity to environmental challenges during adolescence. The latter possibly includes the consequences of accidental exposure to hormones, drugs or environmental pollutants (e.g., Ref. [31]). Our understanding of the complex effects of subtle alterations in hormonal milieu during fetal life on behavioral development, requires further investigation, involving a wider range of behavioral responses at different developmental stages, and possibly in different mammalian species.

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Intrauterine position has long-term influence on brain μ-opioid receptor density and behaviour in mice

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Abstract

In multiparous rodents, a naturally occurring variation in degree of exposure to sex steroids during the prenatal phase of sexual differentiation derives from the in-utero proximity to opposite sex foetuses. So far, the studies on intrauterine position (IUP) phenomenon have mostly focused on traits relating to reproduction and behaviour, while its influence on neurochemical substrates and pharmacological response has been largely unexplored. We investigated possible variations in the function and the profile of expression of the μ-opioid receptor system in three groups of adult mice from known IUP: 2M mice (located between two males), 0M (between two females), and 1M (between a male and a female). Autoradiographic study revealed in female mice that proximity to at least a male in utero (1M and 2M position) resulted associated at adulthood with an increased density of midbrain μ-opioid receptors. Behavioural observations were conducted following injection with the specific μ-opioid agonist Fentanyl (at 0, 0.01 or 0.05 mg/kg IP). A drug-conditioned place preference test confirmed that 1M and 2M subjects were also more sensitive to the rewarding effects of the drug, since mice spent significantly more time in the drug-paired compartment than 0M subjects. In a hot-plate test, 2M subjects showed levels of drug-induced analgesia that were much higher than other IUP groups. No reliable differences were observed between the IUP groups for locomotor

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activity upon drug treatment. Overall, these data indicate for the first time that the organisation of the μ-opioid receptor system in the brain, as well as a differential vulnerability to abuse of opiate drugs can be modulated by epigenetic variables such as the prenatal in utero contiguity to male foetuses.

Keywords: Gonadal hormones; Intrauterine position; Prenatal environment; Behaviour; Opioids; μ-Receptors; Mice

1. Introduction

The development of the central nervous system and behavioural patterns is under the influence of both genetic and epigenetic factors. Sex steroids exert potent influences on the nervous system during critical developmental periods and into adulthood, by organising and reorganising the neuronal circuitry involved in neuroendocrine and behavioural functions (McEwen, 1991. A differential degree of exposure to such hormones during prenatal life is determined genetically by the sex of each subject. However, in all multiparous rodent species examined to date (e.g., mice, rats, and gerbils), the location an individual occupies as a foetus in relation to the sex of its adjacent littermates, has also been shown to account for a significant degree of variation of the hormonal milieu. In mice, proximity to foetuses of the opposite sex determines a 100% increment in testosterone exposure for females, whereas a 50% increment of oestradiol in the case of males (vom Saal and Bronson, 1978; vom Saal, 1983). The individual variation observed in association with intrauterine position has been reported on different behavioural traits not only related to reproduction (vom Saal, 1981; Hauser and Gandelman, 1983; Kinsley et al., 1986; Even et al., 1992; Cologer-Clifford et al., 1992; Howdeshell et al., 1999; Palanza et al., 2001).

A very recent study (Palanza et al., 2001) conducted on adolescent mice of both sexes, reported that male subjects located in utero between two males (2M) expressed a much higher profile of novelty seeking with respect to males located prenatally between two females (0M). Given that the response to novel stimuli and sensations is the basis of the definition of sensation-seeking, individual differences in reactivity to novelty have been studied on behavioural and biological levels (Zuckerman, 1984; Deroche et al., 1993; Resnick et al., 1993; Dellu et al., 1996). These behavioural traits have also been proposed as a predictive factor for vulnerability to abuse of opiate and psychostimulant drugs (Deroche et al., 1993; Laviola et al., 1999) . In this framework, the issue of differential potential vulnerability to drugs of abuse in both adolescent and adult subjects is suggested to be associated with subtle epigenetic changes in hormonal milieu early in development [for the relevance of this in studies in humans see Resnick et al. (1993), McGue et al. (2000), Miles et al. (2001)].

Various observations suggest that a differential uterine position could have an influence on the organisation of μ-opioid system. First, brain sexual differentiation parallels the ontogenesis of this receptor system, and regional distributions of hormonal steroids and μ-opioid receptors are reported to be overlapping (Hammer,

1984). Also, the prenatal rodent and human brain are characterised by the very early expression of μ-opioid receptors with respect to other receptor subtypes, which are mainly postnatal (Rius et al., 1991). Second, there is evidence in mice and rats that sex hormones can modulate the opioid receptor density (Piva et al., 1995; Maggi et al., 1999) within the medial preoptic area, a hypothalamic area whose structural differentiation is known to be primarily an oestrogen receptor mediated function. A work by Hammer (1988) conducted on μ-opioid receptors in the hypothalamus, reported hyper-development in males as a consequence of neonatal treatment with the androgen-receptor blocker flutamide, while exposure to non-aromatisable androgen (dihydrotestosterone) during this period induced hypo-development in females. Moreover, in rodents and non-human primates testosterone is known to regulate pro-opiomelanocortin gene expression in the arcuate nucleus of hypothalamus probably through conversion to oestradiol (Adams et al., 1991; Priest and Roberts, 2000). Third, animals exposed to testosterone in the neonatal period are less responsive to the effects of psychoactive drugs on locomotor activity than were those not exposed to testosterone, whereas effects of morphine are augmented in female rats by circulating oestradiol (Forgie and Stewart, 1993; Stewart and Rodaros, 1999).

Keeping account of these considerations, we addressed the question of the possible modulatory influence of the uterine location on individual variation in the μ-opioid receptor density, as well as the behavioural pharmacological response to administration of a specific μ-opioid receptor agonist. For this purpose two brain areas such as midbrain and striatum, were chosen for examination, since they possess a very high level of μ-opioid receptors and an important role in modulating analgesia and also reward system (Pasternak, 1988; Harrison et al., 1998).

2. Methods

2.1. Subjects

Mice of the outbred CD-1 strain, without prior breeding experience, were purchased from a commercial breeder (Charles River, Italy). On arrival, animals were housed in an air-conditioned room (temperature 21 ± 1 °C, relative humidity 60 ± 10%), with a reversed 12-h light–dark cycle (lights on at 2000 h). Water and food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, Italy) were available ad libitum. To obtain offspring from a known intrauterine position, nulliparous females were time-mated by placing them with a sexually experienced male. Upon discovery of a vaginal plug (designated as gestation Day 0), the females were housed in pairs in Plexiglas cages (33 × 13 × 14 cm) and after approximately two weeks, housed individually.

All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimise suffering, to reduce the number of animals used, and to utilise alternatives to in vivo techniques, if available.

The procedure followed the method described by Palanza et al. (2001). On day

18th of gestation (generally 8 h prior to term), each female was sacrificed by cervical dislocation, a midline incision was made down the ventrum, and the uterine horns were exposed. Each foetus was immediately removed in the order found, gently cleaned with water, sexed, weighed and its intrauterine position recorded. The wet foetuses were next placed under heat lamps to keep warm. Anogenital distance was measured for all the pups using a Zeiss (Germany) microscope with a micrometer lens (accurate to 0.05 mm). One person to whom pups were passed made measurements without knowledge of the intrauterine position. Pups were unequivocally marked using a toe-clipping pattern to allow for identification.

Within 10 min the neonates were next fostered to lactating female mice who had delivered vaginally during the previous 24–48 h. A great effort was devoted to minimise possible cannibalism occurrence following fostering. Specifically, the dam to become a foster mother was temporarily moved to a clean cage. Her pups were carefully removed without altering the nest structure, and the caesarean-delivered pups were placed into the nest and covered with the nest material. Pups were warm and moving when introduced into the nest, and the toe-clipped mark was completely recovered. Litters were culled to four males and four females when possible. The following three groups of animals were then formed: animals from each sex that resided between two females (0M: $n = 13$ females and 25 males), between two males (2M: $n = 25$ females and 23 males) or between a male and a female (1M: $n = 18$ females and 27 males).

Mouse pups were weaned on day 21 and housed according to sex in each Plexiglas cage (33 × 13 × 14 cm). Experiments were conducted at the age of postnatal day (pnd) 70.

2.2. Autoradiography

Brains from saline (SAL) injected animals were removed rapidly onto an ice-cooled metal plate and immediately frozen on dry ice and stored at −70 °C until the time of autoradiographic assays on μ receptors. Analysis was conducted on periacqueductal grey area (PAG) for midbrain, whereas for striatum analysis was pooled over caudate-putamen and nucleus accumbens.

Brains were brought to −18 °C in a cryostat and a series of 12-μm thickness serial sections were collected on gelatine-coated slides. The sections were dried at 30 °C and stored at −80 °C until they were processed for receptor binding autoradiography according to Eghbali et al. (1987). Briefly, slides were incubated for 1 h at room temperature with 4.5 nM [^{3}H]DAMGO (52.5 Ci/mM, NEN Life Science Products, Italy) in binding buffer (50 mM Tris–HCl, pH 7.4, 10 (10 μM bacitracin)). Sections were rinsed three times for 5 min at 4 °C in a 50 mM Tris–HCl (pH 7.4) buffer. After washing, the sections were dipped briefly in water and dried under a cool stream of air. Autoradiograms were made exposing the dried sections in X-ray cassettes for 40 days to a tritium-sensitive film (Hyperfilm-3H; Amersham Italia, Milan, Italy) then developed with a Kodak D19 developer (25 °C, 4 min), fixed in Kodak Unifix (8 min) and rinsed with water (5 min).

The intensity of the receptor binding signal was assessed by measuring the grey

levels of the autoradiographic films with an image analysis system consisting of a solid state video camera (Hamamatsu, Tokyo, Japan) connected to an Apple Macintosh II personal computer. We used the public domain Image 1.47 software (NIH, Bethesda, MD, USA). Each brain section was traced with a mouse cursor control, and the light transmittance was determined as grey level. The grey level of densitometric measurements calculated after a subtraction of the film background density was established within the linear range, determined using tritium standards (^{3}H Microscales, Amersham Italia, Milan, Italy). The mean light transmittance values were obtained by averaging the measurements from autoradiograms of the brain sections from at least three mice. The average light transmittance was converted to femtomoles bound per milligram of wet weight of tissue using the conversion values of tritium standards (^{3}H Microscales Amersham Italia, Milan, Italy).

2.3. Drug treatment

Fentanyl citrate (FEN, Pharmacia Milano, Italy) was injected IP at 1% body weight. The range of doses (see Procedure) and the route of drug administration were chosen on the basis of literature data (Mucha and Herz, 1985).

2.4. Behavioural observations

2.4.1. CPP paradigm—apparatus

The place conditioning apparatus consisted of an opaque Plexiglas rectangular box with smooth walls, subdivided in three different compartments separated by removable walls. Two cues, one visual and one tactile, were associated with each of the two end-compartments, called respectively White (white painted walls and wide mesh floor), and Black (black painted walls and narrow mesh floor). The middle compartment (Middle) was a neutral chamber with grey walls and smooth floor, and served as starting point. The two end-compartments measured 16 × 15 × 30 cm high, while the central one measured 8 × 15 × 30 cm high. The two partitions separating Middle from the end compartments could be replaced by similar partitions with a 4 × 7 cm opening, which allowed free access to all compartments. Each compartment was provided with four infrared photobeams, placed on the wall at few centimetres from the floor. An IBM computer provided with specific software recorded each beam interruption eventually caused by mice. The floor and the wall of the apparatus were washed with a solution of water and ethyl alcohol after each animal was tested.

2.4.2. Procedure

Mice were trained and tested when adults (pnd 70–78), according to the same procedure used in previous experiments [for more detail see Laviola and Dell'Omo (1992) and Laviola et al. (1994)]. The whole experimental schedule took a total of 8 days, each subject being tested between 1000 and 1800 h. On days 6 and 7, animals underwent a washing-out period and were left undisturbed in the animal facility. Testing of different sex and IUP groups was counterbalanced across time, and was

done in a laboratory room isolated from the animal colony, maintained under standard humidity and temperature conditions, and conducted under dim illumination.

2.4.2.1. Phase 1 (day 1, habituation) Inexperienced mice were placed singly in the Middle compartment with free access to all compartments, and allowed to explore the apparatus for 10 min. No formal record of individual preference was kept.

2.4.2.2. Phase 2 (days 2–4, conditioning) Subjects from both sexes and IUPs were randomly assigned to one of three treatment groups (0, 0.01, or 0.05 mg/kg of IP Fentanyl). On the first day of the conditioning phase, animals were injected and confined immediately after in the assigned (paired) end-compartment for 30 min. Twent-four hours later, all subjects received a saline injection before being confined in the opposite (un-paired) end-compartment. The same procedure was used for the 3rd and the 4th day of the conditioning phase. Data on locomotor activity, measured as number of photobeams interruptions/s were collected automatically during days 2 and 4 of the conditioning phase.

2.4.2.3. Phase 3 (day 8, CPP test) CPP assessment (drug-free state) was conducted 72 h after the last exposure in the Phase 2 by placing uninjected mice in the Middle compartment and allowing them free access to both end compartments for 10 min. The following measures were obtained automatically: locomotor activity in each compartment (number of beam interruptions/s); time spent in each compartment, and total locomotor activity, (number of transitions from the Middle to the end-compartments).

2.4.2.4. Nociception assessment One week after the CPP test, animals were assessed for pain reactivity in a hot-plate apparatus (Socrel Hot-Plate model-DS37: Ugo Basile, Italy) and analgesia measured as latency for hind-paw licking. Temperature was set at 55 ± 1 °C, with cut off being set at 60 s. Mice were administered the same drug dose received during the conditioning phase of the CPP schedule (see Procedure) and were gently placed 15 min after, on the hot-plate apparatus.

2.5. Design and statistical analysis

Data were analysed by using parametric analysis of variance (ANOVA) with two levels of sex, three levels of IUP, and three levels of drug as between-subject factors. Days were considered as repeated measures factor (Chiarotti et al., 1987; Winer, 1971). Separate analyses were performed when allowed by the finding of a significant main effect in the ANOVA. Multiple comparisons were performed using the Tukey HSD test. Correlations between receptor density and measures from the nociception test, were analysed by Pearson's linear correlation coefficient. Statistical significance was set at 0.05.

3. Results

3.1. Effects of IUP on μ-receptor density

Following behavioural analysis, brains of SAL-injected subjects were removed for autoradiographic binding studies in order to evaluate sex differences as well as potential IUP related differences in basal μ-receptor density. Analysis was conducted on midbrain and striatum (see Methods).

3.1.1. Midbrain

ANOVA revealed a main effect of sex, $F_{(1,46)} = 25.26$, $P < 0.001$, with males presenting higher receptor density than females. A main effect of IUP, $F_{(2,46)} = 8.56$ $P < 0.001$, and also a significant sex by IUP interaction, $F_{(2,46)} = 13.65$ $P < 0.001$, revealed an increment in receptors levels associated with the presence of at least one male in utero. This profile was particularly evident in females with 0M position presenting significant lower values than 0M males and other uterine groups ($P < 0.01$). Male subjects from the three IUP groups did not show reliable differences among each other (see Fig. 1 left panel).

3.1.2. Striatum

In this area females were characterised by a higher receptor density than males (main effect of sex, $F_{(1,78)} = 31.98$ $P < 0.001$). Also a marked difference between the three IUP groups was observed. A main effect of IUP, $F_{(2,78)} = 21.38$ $P < 0.001$, together with a significant sex by IUP interaction $F_{(2,78)} = 7.25$ $P < 0.001$, indicated that in males an increased receptor density was associated with the conti-

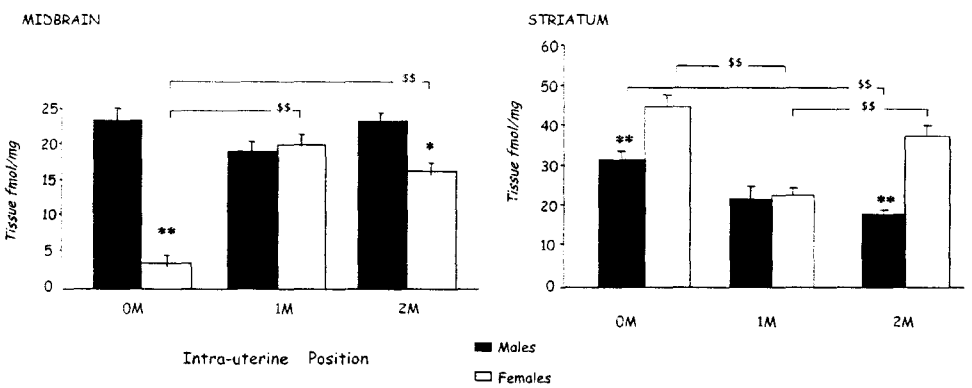

Fig. 1. Long-term effects of IUP on μ-receptor density shown in mice of both sexes. Data are derived from SAL-injected mice only, and are expressed as femtomols bound per milligram of wet weight tissue. Midbrain (left panel): $*P < 0.05$ for 2M females vs 2M males and $**P < 0.001$ for 0M females vs 0M males; $^{ss}P < 0.01$ 0M vs other IUP conditions within the female group. Striatum (right panel): $**P < 0.001$ for 0M females vs 0M males and 2M females vs 2M males. $^{ss}P < 0.01$ in multiple comparisons performed among the IUP conditions within each sex.

guity to opposite-sex foetuses in utero (see Fig. 1 right panel). In females contiguity to only a male (1M condition) markedly reduced receptor density.

3.2. Behavioural analysis

3.2.1. Activity on days 2 and 4 (conditioning phase)

Overall, no sex differences were observed for this parameter ($F_{(2,112)} = 0.42$ $P = 0.51$ n.s). ANOVA on activity data collected over the two days of training revealed a significant main effect of drug administration, $F_{(2,112)} = 56.88$ $P < 0.001$. As a whole, a dose-dependent reduction of activity in drug-injected mice was found when compared to the SAL group ($P < 0.001$ for SAL vs the FEN-0.01 and FEN-0.05 group). In the absence of significant differences in the baseline (SAL-injected mice) among animals belonging to the three IUP groups, a long-term influence of IUP was found in response to the drug challenge, with a IUP by drug by day interaction ($F_{(4,112)} = 2.95$ $P < 0.05$). Specifically, 1M and 2M mice exhibited a persistent and significant reduction of activity at the low (1M) and high (1M and 2M) FEN-dose during both days of training (see Table 1). No reliable or significant differences were observed between the IUP groups upon FEN treatment.

3.2.2. CPP-time spent in the paired compartment on day 8 (testing day)

No carry-over influence of previous Fentanyl treatment was observed (drug effect, $F_{(2,112)} = 0.08$ $P < 0.92$, n.s). Male subjects spent as a whole more time in the paired compartment with respect to females (main effect of sex, $F_{(2,112)} = 7.22$ $P < 0.01$). A main effect of IUP was also found, $F_{(2,112)} = 7.64$ $P < 0.001$, indicating that 0M subjects spent significantly less time in the paired compartment than the other IUP groups (165 vs 199 and 183 s, $P < 0.001$ 0M vs 1M). This profile was more evident in the female group (sex by IUP interaction just missing significance $F_{(2,112)} = 2.90$ $P = 0.058$, for post-hoc comparisons see Fig. 2).

3.3. Hot-plate test

For the latency to lick a hindpaw, a main effect of drug in the ANOVA ($F_{(2,111)} = 52.16$ $P < 0.001$) revealed a dose-dependent analgesia profile (see Fig. 3). As a whole, no sex differences were observed for this parameter (sex effect, $F_{(1,111)} = 2.59$ $P = 0.10$; sex by IUP interaction $F_{(2,111)} = 0.04$ $P = 0.95$, n.s). In the absence of significant differences in the baseline (SAL-injected mice) among animals belonging to the three IUP groups (ANOVA, $F_{(2,111)} = 2.03$ $P = 0.13$ n.s), a IUP by drug interaction was also found, $F_{(4,111)} = 2.48$ $P < 0.05$. Specifically, 2M subjects resulted the most responsive to the drug since a maximal level of analgesia was reached in this group already at the low FEN-0.01 dose ($P < 0.01$). In contrast, the same drug dosage was apparently ineffective in the 0M group. Interestingly, the response to the low FEN dosage was progressively higher in the 1M and 2M groups. This difference reached the significance in the comparison between 0M and 2M mice ($P < 0.05$).

Table 1
Long-term effects of IUP on drug induced locomotor activity (SEM) on days 2 and 4 of the conditioning phase (CPP paradigm). Locomotor activity was measured automatically as the number of photobeams interruptions/s during a 30-min session/day, in adult mice belonging to the three IUP conditions. Mice were injected IP either with SAL, FEN-0.01 or FEN-0.05 and immediately placed in the drug-paired side of the apparatus. Data are collapsed over the sexes ($n = 8-18$)

IUP		0M	1M	2M
DAY 2	SAL	338.68 (19.59)	382.45 (24.24)	316.04 (23.46)
	FEN-0.01	225.56 (47.69)	143.50 (17.89)**	198.26 (29.95)**
	FEN-0.05	138.40 (27.58)**	124.23 (32.05)**	116.87 (26.31)**
DAY 4	SAL	299.13 (24.67)	337.96 (25.19)	311.40 (28.85)
	FEN-0.01	188.12 (43.78)	291.37 (42.85)**	225.56 (28.69)
	FEN-0.05	155.74 (28.77)**	141.20 (22.73)**	109.21 (24.43)**

** $P < 0.001$ for FEN-0.01 and FEN-0.05 vs SAL within each IUP group on days 2 and 4 (Tukey HSD test).

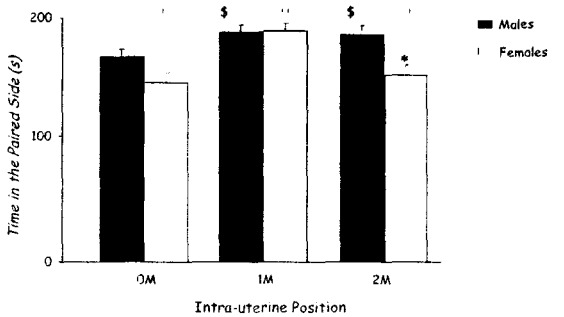

Fig. 2. Long-term effects of IUP as measured in the CPP test by mice of the two sexes. Data represent mean (±SEM) time spent in the paired compartment of the apparatus during a single 10-min session by adult mice on testing day 8 (drug-free state), and are presented pooled over the Drug variable. $^{\$}P <$ 0.05 within female's IUP groups. $*P < 0.05$ between 2M females vs 2M males.

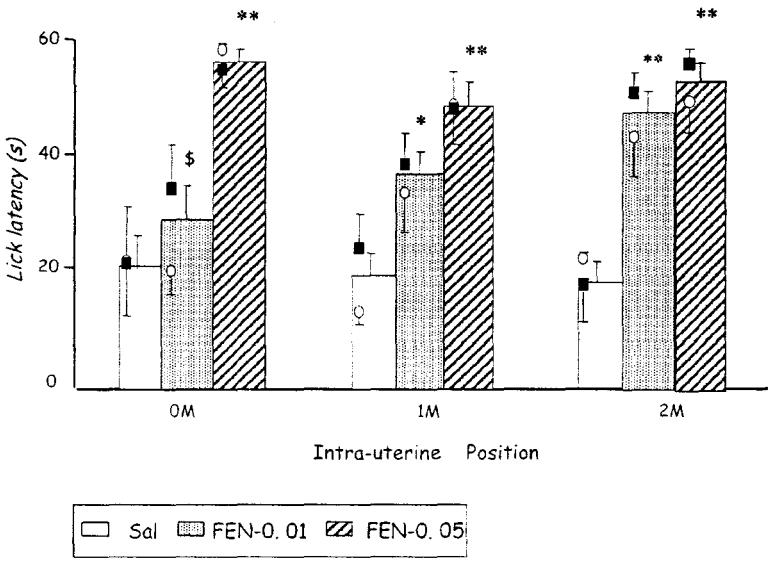

Fig. 3. Long-term effects of IUP as measured in the hot-plate test (55 ± 1 °C). Hindpaw lick latency (mean ±SEM) shown by adult mice of both sexes injected either with SAL, FEN-0.01 or FEN-0.05, 15 min before testing (empty circles females, filled squares males). $*P < 0.05$ between SAL vs FEN-0.01 within the 1M group. $**P < 0.01$ between SAL vs FEN-0.05 and vs FEN-0.01 within the 0M and the 2M groups; and SAL vs FEN-0.05 within the 1M group. $^{\$}P < 0.05$ between 0M and 2M subjects within the FEN-0.01 treatment group.

3.4. Correlation analysis

Measures from the hot-plate test of SAL-injected mice were then analysed separately for possible correlations with receptor density.

Midbrain: no linear correlation was found in both sexes. Striatum: no correlation

was found for females ($r = -0.02$ $P = 0.93$) whereas a negative correlation was found for males ($r = -0.60$ $P < 0.01$).

A second analysis was carried out separately for each sex considering the IUP variable. Midbrain: a positive correlation was observed for females ($r = 0.94$ $P < 0.05$; $r = 0.85$ $P < 0.05$ and $r = 0.84$ $P < 0.01$, for 0M, 1M and 2M groups respectively). For males no correlations were observed in the 0M and 1M groups ($r = 0.84$ $P = 0.07$ and $r = 0.74$ $P = 0.46$, respectively) whereas a negative one was found for 2M group ($r = -0.93$ $P < 0.01$).

Striatum: no linear correlation was found for females IUP groups ($r = -0.78$ $P = 0.11$; $r = -0.80$ $P = 0.56$; $r = -0.65$ $P = 0.078$). In contrast, a negative correlation was observed within male subjects for 0M and 1M groups ($r = -0.98$ $P < 0.01$, $r = -0.85$ $P < 0.01$), whereas a positive one for the 2M group ($r = 0.92$ $P < 0.01$).

4. Discussion

The main findings can be briefly resumed as follows:

1. The autoradiographic μ-receptor analysis revealed in the midbrain area marked sex differences with males presenting higher number of μ receptors than females. This analysis also evidenced that female mice, which were located in utero between male foetuses, presented an increased receptor density than other IUP groups. In the striatum, females presented higher density than males. Interestingly, females contiguity was associated with an increased receptor density in males.
2. Acute administration of FEN, a direct μ-opioid receptor agonist induced a dose-dependent reduction of activity levels, with adult mice belonging to the 1M and 2M condition being the most responsive to the drug challenge.
3. In the CPP test, 1M and 2M mice appeared also more sensitive to the rewarding effects of the drug, since they spent more time in the drug-paired compartment than 0M subjects.
4. Long-term influences of IUP were particularly marked in the FEN-induced analgesia with 2M subjects being significantly more responsive to the drug than the 0M group upon the same drug dosage.

In the present study, a significant within sex variation clearly emerges in adulthood as a function of contiguity to male foetuses in utero. The latter has been associated in the literature to increased concentrations of male-derived hormones (vom Saal and Bronson (1978).

A sexual dimorphic profile was observed in the midbrain, specifically in the periaqueductal grey that is known to be principally involved in the regulation of pain perception and in the modulation of analgesic responses. In particular, a higher density of μ receptors was found in males than in females. This profile is consistent with previous literature data indicating higher basal threshold to pain stimulation and

higher morphine-induced analgesia in males than in females (Beatty, 1979; Forman et al., 1989; Kavaliers and Colwell, 1991). The literature on this topic is however mixed since apparent contrasting results are also available (see Hammer, 1988; Tershner et al., 2000).

Furthermore, with respect to the IUP modulatory influence on this sex-genetically determined profile, an increasing gradient in receptor density in association with the number of contiguous male foetuses in utero (0M<1M<2M) was clearly evidenced within the female group. In this line, radioimmunoassay studies conducted by vom Saal and Bronson (1978), have shown that location intra-utero between male foetuses is a condition susceptible of increased exposure to testosterone (see Introduction). On the other hand, no reliable changes were observed within the male group. It is possible that the higher levels of testosterone, to which males but not females are naturally exposed, were such that subtle changes in μ-opioid receptor density in the midbrain related to uterine-location were not detectable.

Data from the correlation analysis are worthy of several comments. When analysed separately for each sex and by IUP variable, a positive correlation between receptor density in the midbrain and lick latency in the hot-plate test was observed in all female's IUP groups. This general profile is not evident when considering the baseline hot-plate response in the three IUP groups which did not differ between the sexes. However, it is consistent with the significant differences observed within the IUP groups upon FEN-induced stimulation of the μ-opioid system.

The long-term effects of IUP found in the midbrain of both males and females are consistent with a wide range series of behavioural results reported in the present study. In general, this set of data clearly indicates an increased sensitivity to a challenge with Fentanyl a selective μ-opioid agonist in adult 2M individuals when compared to other groups.

In fact, in this IUP group, acute administration of FEN induced a marked dose-dependent reduction in locomotion. Also, when mice were assessed in a conditioned place preference paradigm for sensitivity to the FEN-related positive reinforcing properties, significant differences between IUP groups were found. Again, mice belonging to the 1M and 2M condition (i.e., subjects exposed prenatally to male foetus-derived hormones) were associated with longer time spent on testing day (drug-free state) in the specific compartment of the apparatus which was paired with drug effects during the conditioning phase of the CPP paradigm. This evidence indirectly suggests an increased sensitivity to drug effects in these two phenotypes. This profile is also in strict accordance with the results obtained from the nociception assessment (see Fig. 3). In this paradigm, upon the same drug dosage increasing levels of FEN-induced analgesia in adult subjects were functions of the increasing number of contiguous male foetuses.

With respect to the striatum—a brain region with a strong involvement in the modulation of locomotor activity and the reward system (see Pasternak (1988); Harrison et al. (1998)—the receptor profile evidenced in the present study appears in agreement with previous findings concerning a direct and opposite influence of oestrogens on the binding characteristic of brain opioid receptors (Weiland and Wise, 1990; Zubieta et al., 1999). In fact, adult female mice exhibited in general higher

receptor density than males. Within the male group prenatal contiguity to females, which represents a condition susceptible of increased exposure to oestradiol, was associated with an increasing gradient in μ receptor density (2M<1M<0M). No additional reliable changes related to IUP were observed within the female group, probably because of the higher levels of oestradiol, to which females but not males are naturally exposed. Interestingly, contiguity to only a male—1M condition—but not two (2M condition) markedly reduced receptor density, thus determining a U-shaped profile for the three IUP groups (0M>1M<2M) in the striatum area. However, the contribution of the 1M uterine position to the exposure to a different gradient of sexual hormones during prenatal life remains yet to be fully determined and measured (vom Saal and Bronson, 1978).

An interesting finding that emerges from our study is that effects of IUP on organisation profile of μ receptors vary in the two sexes as a function of the brain area involved. Specifically, whereas in the midbrain receptor profile in the two sexes parallels the behavioural profile, dissociation between the sexes is observed for the striatum. In fact, only female data were consistent with behavioural measures. In this view, it should be noted that locomotor activity is a sexually dimorphic behaviour with typical higher levels shown in females than in males. Interestingly, females have also been reported to be more precocious and generally more responsive than males in the ontogenetic analysis of the effects of drugs of abuse (see Laviola et al., 1994). Furthermore, it must be taken into account that apparent inconsistencies in IUP effects probably reflect differences in the way slight changes in testosterone and oestradiol levels interfere with basic regulatory mechanisms during development in the two sexes (see Kinsley et al., 1986; Howdeshell et al., 1999).

To our knowledge, this study provides a first evidence that an epigenetic variable such as the IUP phenomenon namely developing next to a male foetus in utero, has important long-term consequences on brain/behavioural functions. Specifically, in the brain of adult animals we observed a differential μ-opioid receptor organisation as well as a differential sensitivity to a specific psychotropic agent targeting to this system. In this framework, the understanding of the complex effects of subtle alterations in hormonal 'milieu' during foetal life on behavioural and neuroanatomical development would involve a better comprehension of the individual variability in coping with environmental challenges including pain perception as well as a differing sensitivity to analgesic compounds reported in clinical literature. The issue of differential potential vulnerability to drugs of abuse in both adolescent and adult subjects could also be investigated in light of subtle changes in hormonal milieu early in development, such as those associated with the IUP phenomenon.

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Soumis à European Journal of Pharmacology

Prenatal stress affects MDMA pharmacokinetics as well as drug-induced behaviour in adolescent rats

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Abstract

Prenatal stress (PS) has been shown to enhance sensitivity to drugs in adult rats. In addition to early experiences, the adolescent period is associated in both animals and humans with an increased risk in developing drug abuse. Following a single oral administration of 3,4-methylenedioxymethamphetamine (MDMA), adolescent female PS rats presented an increased balance skill impairment on a runaway paradigm. Moreover, PS had also a long-term effect on the metabolic rate of MDMA which resulted constantly higher in the PS group during the kinetic assessment when compared to control subjects. These results provide evidence of a higher vulnerability to drugs in PS animals observable early at the adolescent stage, and further indicate that early differences in metabolism can have a profound impact on the effects to drug abuse.

INTRODUCTION

Environmental factors may play a key role in determining individual variability to psychostimulants. In this regard, stress-induced corticosterone secretion may play a role in determining individual differences in sensitivity to psychostimulants drugs (Piazza et al., 1991) and there is evidence that repeated exposures of adult animals to stressful conditions increase individual vulnerability to psychostimulants (Piazza and Le Moal, 1996). In rats, exposure to stress during prenatal life affects pharmacological systems that are particularly relevant for the study of drug abuse. Indeed, prenatal stress (PS) has been found to result in functional alterations of the mesolimbic system (Henry et al., 1995) and enhanced propensity to self-administer amphetamine (Deminiere et al., 1992) as well as increased locomotor reactivity following nicotine administration (Koehl et al., 2000) has been reported in PS adult subjects.

Up to date, the major part of the studies conducted on animal models and drugs of abuse, have been mostly carried out on adult subjects although an increased drug use and a higher risk to develop drug-related problems is often observed already at the adolescent period, during which different patterns of temporary deviance are often observed (Laviola et al., 1999). The animal model of adolescence in the rodent, validated by (Spear and Brake, 1983), covers the whole postnatal period ranging from weaning to adulthood (21-60 days) and represents a useful tool for investigating the vulnerability to a variety of habit-forming agents or emotional experiences whose positive reinforcing properties may rely on common neurobiological substrates (Laviola et al., 1999). Subjects around this age exhibit elevated levels of novelty seeking behaviour together with a peculiar sensitivity to administration of psychostimulant agents (Laviola et al., 1995;Adriani et al., 1998). In fact, adolescent subjects exhibit a reduced sensitivity to various drugs of abuse and such insensitivity can promote greater use per occasion relative to more mature individuals (Spear, 2000).

3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is an illicit drug with significant abuse potential (Cami et al., 2000). Likewise, several disturbances widespread at the behavioural and neurochemical level and ranging from wanted effects like euphoria, central

nervous stimulation to impairment of cognition and motor co-ordination are being increasingly recognised in association with MDMA abuse. (Parrott et al., 1998) (Schifano et al., 1998). (Gerra et al., 2002) It has been assumed that the risk of being involved in fatalities and accidents during the state of MDMA influence is increased. Indeed, observations of the prevalence of MDMA involvement in cases of reckless driving and the MDMA blood concentrations measured, indicate a risk increase comparable to that observed after use of amphetamines (Morland, 2000).

In the present study, we assessed animals' performance in a simple motor coordination task following oral administration of MDMA in adolescent female rats. A measurement of MDMA levels in the blood was also conducted at different time points after the oral administration procedure, to investigate the kinetics of MDMA, and also to compare these endpoints to any behavioural effects produced by the drug.

MATERIALS AND METHODS

Animals and breeding

Sprague-Dawley female rats weighing approximately 250 g without prior breeding experience, were purchased from a commercial breeder (Charles River, Italy). Animals were housed in an air-conditioned room (temperature 21±1°C, relative humidity 60±10%), with a regular light/dark cycle (lights-on at 8.00 p.m.). Water and food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, Italy) were available *ad libitum*. Females were placed with a sexually experienced male and daily inspected for vaginal smear until the discovery of spermatozoids (designated as day of gestation 0), after which they were housed individually in Plexiglas cages (30x20x15 cm). Pregnant females were then randomly assigned to Prenatal stressed (PS) or Control (CONT) groups.

Prenatal stress

Stress procedure started on day 11 of pregnancy until delivery at 21 days according to Maccari and coworkers (Maccari et al., 1995) as follows: pregnant females were individually placed in plastic transparent cylinders (7 cm diameter, 19 cm long) and exposed to bright light for 45 min. Animals were submitted daily to three stress sessions starting at 09:00, 12:00 and 17:00 h, whereas control pregnant females were left undisturbed in their home cages. Male and female offspring were weaned on day 22 after birth, and only offspring from litters containing 10-14 pups with a comparable number of males and females were used in the experiments. After weaning, animals from each experimental group were housed in same-litter groups of five and maintained in the same environmental conditions as their mothers until experiments started. In the present study only females offspring of the control and PS group were used.

Drug and drug administration

MDMA (Lipomed, Switzerland) was diluted in water to provide the appropriate dose (5 mg/kg) and administered orally 1% of body weight. Dosage was selected on the basis of literature (Miczeck and Haney 1994). Adolescent female rats (pnd 30-32) from control and PS group were housed in group of five per were weighed and gavaged once with either water (VEH subjects) or 5 mg/kg MDMA dissolved in distilled water. VEH subjects were scored immediately after the gavage procedure, whereas MDMA treated subjects were scored at different time points such as 15, 60, 120 or 180 minutes after the gavage. Within litter, each rat was randomly assigned to one of the five time points after treatment (t_0, t_{15}, t_{60}, t_{120} and t_{180}).

Motor test

Rats were trained to traverse a straight Plexiglass runway, 5x100cm suspended 1m above the floor. The apparatus was located in a sound attenuated room, and the test conducted under dim light. The test consisted of two trials of 2 min each with a one-minute inter-trial. The animal was gently placed on one end side (departure) and the following behavioural measures were taken: episodes and time spent inactive on the string, and measures of balance skill impairment represented by frequency of slips (the animal slips on the string with one fore paw) and of twists (the animal turns completely on its body with the four paws).

Pharmacokinetic examination of MDMA blood levels

At the end of the behavioural tests, animals were sacrificed by decapitation and their trunk blood collected in heparinised vials. MDMA concentration was then measured by headspace solid-phase microextraction tandem gas chromatography-mass spectometry (Gentili et al., 2002). This method provides good sensitivity and specificity with limits of detection and of quantitation below 2 ng/mg and 4 ng/mg, respectively. Intra- and inter-assay precision were within 2% and 9 % respectively. Blood sample (50µl) didn't require any treatment.

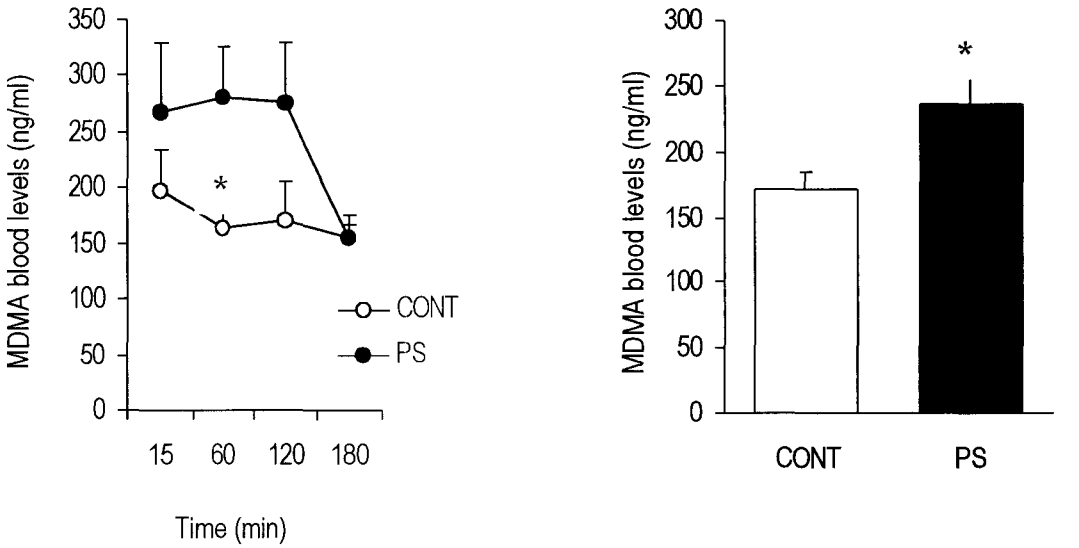

Fig. 1 Pharmacokinetics of MDMA blood levels shown by CONT (n=6) and PS (n=6) at different time points after gavage procedure.

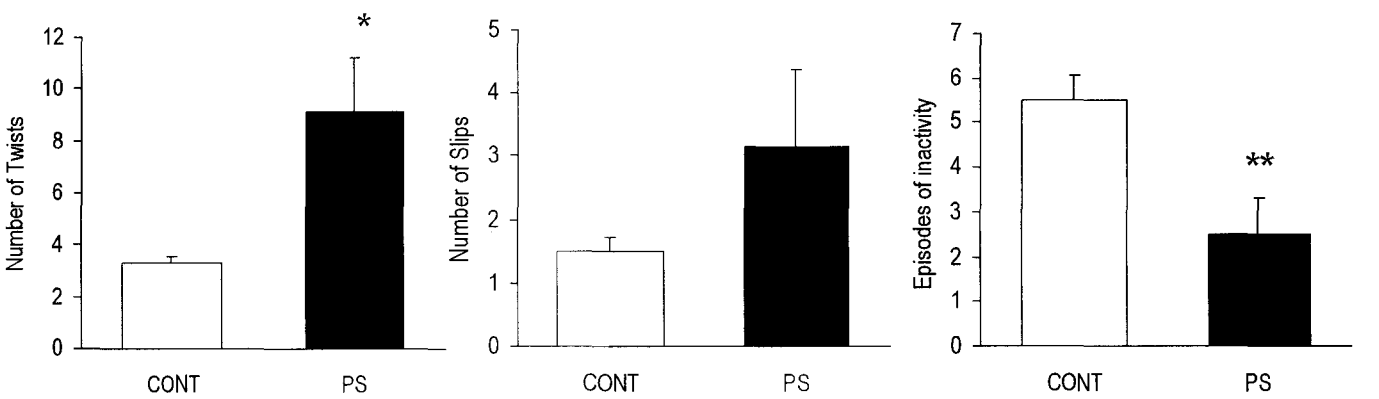

Figure 2. Performance test. Mean number (SEM) of twists, slips and episodes of inactivity on the string shown by control (CONT, n=6) and prenatal stress (PS, n=6) groups. *p<.05, and **p<.01 PS vs NS group.

Statistics

Data were analysed using parametric analysis of variance (ANOVA) with two levels of group (controls vs PS) as between group variable and five time points (t_0, t_{15}, t_{60}, t_{120} and t_{180}) as within group variables. Student's t-test was used for comparisons.

RESULTS

MDMA levels in the blood

Results are presented in fig. 1. PS rats presented higher values of circulating MDMA than controls (main effect of group ($F(1,8)= 5.87$, $p< 0.05$). A trend toward time dependent decreasing levels of MDMA was also observed (main effect of time just missing significance ($F(3,24) = 2.62$, $P = 0.07$). The difference reached significance at t60 after gavage administration (Student's t-test, $t = -2.54$, $P< .05$).

When considering the composite percentage of individuals that exhibited each a discrete value, 60% of subjects in the control group accounted for 0.1-0.2 µg/ml of MDMA with respect to just 40% of individuals in the PS group. The highest values were found in the PS group with almost 20% of individuals measuring 0.4-0.5 µg/ml, whereas no control subjects were found for such value (Pearson Chi-square = 8.45, P<.03). Furthermore, the 0.1-0.2 µg/ml range of MDMA levels was represented by most of the subjects throughout the measurement period (between 66 and 40% for NS and PS groups respectively). When considering the time course of such value, a peak was found at t60 and t180 in control group with 100% of individuals in each point, whereas 40 and 60% of subjects were observed in each point for PS group. In an inverted U-shaped profile with a peak at t60 (65%), the high concentration range was reached in the PS group (35%) already at t15.

Motor test

Results are presented in fig. 2. ANOVA yielded a main effect of group for number of twists and episodes of inactivity ($F(1,9) = 4.94$, $P < 0.05$ and $F(1,9) = 10.48$, $P < 0.01$ respectively), with PS group making more twists than control group, whereas the profile was opposite for episodes of inactivity. With respect to slips a trend toward a two-fold number of slips shown by PS group was found. A group just missing significance ($F(1,9) = 2.15$, $P = 0.07$) revealed a tendency for PS group to present an increased number of slips with respect to control rats.

DISCUSSION

The current study provided pharmacokinetics data on the temporal pattern of drug concentration in the blood of adolescent rats following a single oral administration of MDMA. It is important to note that the present data are not the same as data collected in a typical pharmacokinetics study done in adult animals in which repetitive samples are taken on the same subject over time. Such a within-subjects design, was not feasible in our experiment with early adolescent rats because their blood volume does not accommodate multiple sampling procedure. Moreover, repeated sampling on the same animal would have interfered on its following behavioural performance. Therefore, the present study was a between-subjects design. An effort was made to control for litter effects by ensuring that no two offspring from the same litter were sampled at any single time point but rather, litter mates were used for each time point.

A main finding of the present study was that the experience during prenatal life of a stressful condition had a long-term effect on the metabolic rate of MDMA, in that PS rats showed constantly higher levels of plasmatic MDMA during the kinetic assessment when compared to controls. In this regard, the constant recovery of MDMA in this group seems to point towards a saturation or an inhibition of MDMA metabolism, that could be supposed to occur at the level of the demethylation step (de la Torre et al., 2000). In fact, one of the elimination pathways of MDMA is demethylation to dihydroxymethamphetamine (Hiramatsu et al., 1990). This reaction is mediated via the cytocrome P-450 2D6 in humans and is expressed polymorphically (Hiramatsu et al., 1990). Approximately 10% of Caucasians lack the functional activity of this enzyme and are classified as poor metabolisers of substrates of this CYP isoform (Gonzalez et al., 1988). The remainder of the population is classified as extensive metabolisers. It has been proposed that a deficiency in this enzyme may result in substantially impaired elimination of MDMA, leading to higher and sustained concentrations of the parent drug in the body and increased toxicity (Tucker et al., 1994); (Wu et al., 1997; Ramamoorthy et al., 2002). It is important to note that, differences in the metabolism of MDMA have been reported also in rat strains, with the corresponding enzyme being CYP2D1 (Al Dabbagh et al., 1981). In this regard, it has been shown that the female Sprague Dawley and Dark Agouti rats are the animal counterparts of the human extensive and

poor metaboliser phenotype respectively, since the female Dark Agouti rat is deficient in CYP2D1 (Chu et al., 1996). Although this hypothesis requires certainly further investigation, the different degradation route observed in the PS rats could then lead to suppose an altered activity in the hepatic enzyme CYP2D1. This could also contribute to explain the enhanced sensitivity to drugs reported in adult PS rats (Deminiere et al; 1992; Koehl et al., 2000), in addition to the reported functional alterations at the levels of the mesolimbic (Henry et al; 1995) and serotonergic systems (Peters, 1986; 1990).

Given the importance of drug metabolism in determining the magnitude of the effects of a drug (de la et al., 2000b), the differences in MDMA metabolism observed may impact the likelihood of adverse consequences in particular drug-using individuals such as PS rats. One direct consequence would be the development of acute toxicity at moderate doses of MDMA because the drug would accumulate in the body instead of being metabolised and inactivated. In the present study, a higher frequency of altered motor coordination following MDMA administration was evidenced in PS rats than in control animals, thus indicating a strong consistency between drug blood levels and behaviour.

A potential critical issue in the development of an appropriate animal model relates to pharmacokinetics differences between the species, especially with respect to the half-life of many of the psychostimulants (Cho et al., 2001). Species–dependent elimination rates become even more significant with repeated drug administration, because of differences in the rate and the degree of drug accumulation as a function of interval between successive exposure. Moreover, it is possible that pharmacokinetics characteristics may be altered by prior exposure to the drug. All these factors should be considered since they profoundly influence the behavioural and neurochemical effects of exposure to psychostimulants. In our study, the blood concentrations of MDMA in adolescent rats were already within the range reported following a single MDMA administration in humans (Helmlin et al., 1996), thus supporting the periadolescent rodent as a valid animal model to be used in the investigation of vulnerability to drugs of abuse.

As a whole, these findings indicate that prenatal stress play a crucial role in affecting multiple levels of the individual response to drugs and in addition provide a useful behavioural model for investigating functional consequences of MDMA administration during adolescence.

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CHAPITRE 2

HIGH CORTICOSTERONE LEVELS IN PRENATALLY STRESSED RATS PREDICTIMMOBILITY BEHAVIOUR IN THE FORCED SWIM TEST. EFFECTS OF A CHRONIC TREATMENT WITH TIANEPTINE.

Morley-Fletcher S., Darnaudery M., Koehl M., Munoz C., Casolini P., Van Reeth O., Maccari S.

Soumis à Brain Research

CHRONIC TREATMENT WITH IMIPRAMINE AFFECTS BEHAVIOR, HIPPOCAMPAL CORTICOSTEROIDS AND CORTICAL 5-HT1A RECEPTOR EXPRESSION IN PRENATALLY STRESSED RATS

Morley-Fletcher S., Darnaudery M., Mocaer E., Froger N., Lanfumey L., Laviola G., Casolini P., Zuena A., Hamon M., Maccari S.

En préparation

Le but principal de cette partie est d'évaluer la "predictive validity" du SP chez le rat comme modèle animal de dépression. Chez l'homme, les effets cliniques des antidépresseurs n'apparaissent pas avant deux ou trois semaines après le début du traitement. Pour cette raison, dans les deux études, les rats SP reçoivent des antidépresseurs de fan chronique pendant trois semaines.

Dans la première étude, nous avons évalué si la dysfonction de l'axe HHS pourrait prédire un comportement altéré dans le test de la nage forcée, un test classiquement utilisé pour valider l'activité antidépresseur 5porsolt, 1978). Les animaux étaient traités avec le nouvel antidépresseur (tianeptine) et évalués dans le test de la nage forcée à la fin du traitement.

Dans la seconde étude, nous avons étendu l'évaluation de la "predictive validity" des rats SP en se focalisant sur les effets d'un traitement chronique avec de l'imipramine sur le comportement anxieux, l'axe HHS et le système sérotoninergique.

- Nous avons trouvé une corrélation positive entre les taux de corticostérone et la performance comportementale dans le test de la nage forcée. Les rats SP caractérisés par une hyperactivité de l'axe HHS passent plus de temps en comportement passif par rapport aux contrôles. Aprés le traitement chronique avec la tianeptine, le comportement d'immobilité est réduit de façon marquée.
- Les rats SP étaient caractérisés par des hauts niveaux de comportement de "*self-grooming*" (toilettage) lorsqu'ils se trouvent en présence d'un congénère et par une

diminution de l'exploration des bras ouverts dans le test en labyrinthe en croix surélevé, confirmant ainsi le profil général d'anxiété de ces animaux. Le traitement chronique à l'imipramine à un faible effet anxiolytique dans le test d'interaction sociale mais n'a pas d'effet sur la réponse au labyrinthe en croix surélevé.

- Lorsqu'ils sont évalués dans le test de la nage forcée, les rats SP sont plus immobiles que les contrôles. Après un traitement à l'imipramine, le comportement d'immobilité était fortement réduit.
- La normalisation de l'axe HHS et du système 5-HT par l'imipramine était concomitante à l'augmentation des niveaux de corticostéroïdes hippocampiques et une diminution des niveaux d'ARNm de 5-HT1A, ce qui est en accord avec l'efficacité de la pharmacothérapie chez l'homme.

Effets d'un traitement antidépresseur sur la neurogénèse chez les rats SP (étude en cours dans le laboratoire de Stress Périnatal)

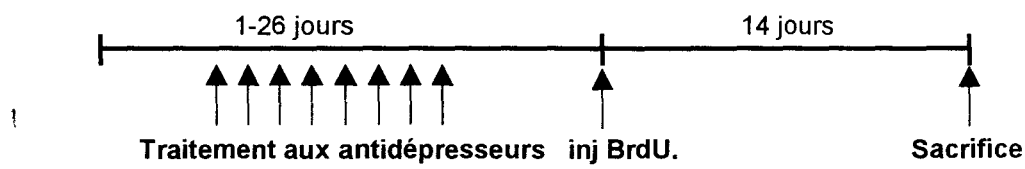
Les expériences stressantes suppriment la formation des cellules granulaires de l'hippocampe chez un gret nombre d'espèces de mammifères (Gould et al., 1998). Cela a été partiellement expliqué par la rétraction des dendrites et la diminution de la prolifération cellulaire (Mc Ewen 1999; 2000). Le traitement chronique aux antidépresseurs peut contrecarrer l'action du stress sur la morphologie et la prolifération des neurones hippocampiques (Watanabe et al., 1992; Malberg et al., 2000; Czeh et al., 2001). Etant donné que le SP a été décrit comme induisant une diminution de la neurogénèse dans le gyrus denté de l'hippocampe, nous avons conduit une étude visant à évaluer les effets d'un traitement chronique aux antidépresseurs sur la neurogénèse hippocampique chez les rats SP. Dans cette expérience, nous avons utilisé un nouvel agoniste de la mélatonine (le S20098, fourni par Servier, France)

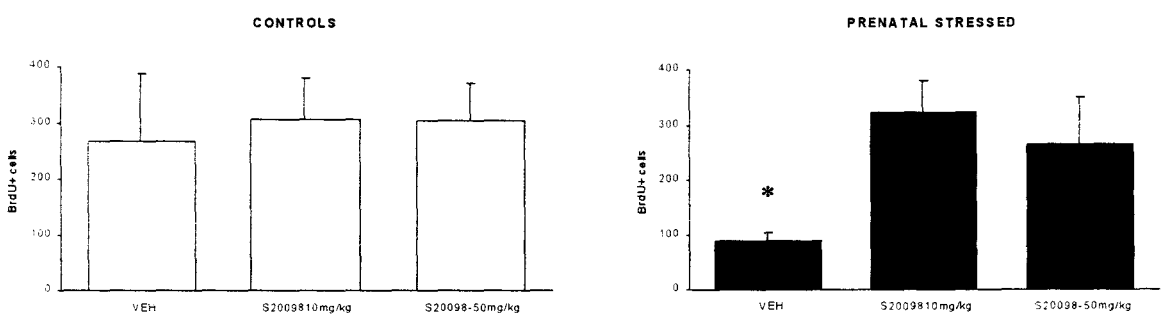
Méthodes : Des rats mâles SP ont été injectés de façon chronique pendant trois semaines avec 0, 10 ou 50 mg/kg de S20098. A la fin du traitement, les animaux sont injectés 4 fois pendant une journée avec l'analogue de la thymidine bromodeoxyuridine (BrdU, 75 mg/ kg i. p.) pour marquer les cellules en division, puis sont tués par perfusion 2 semaines plus tard. Ces deux semaines permettent la détection de la prolifération et de la différenciation de cellules nouvelles (Malberg et al., 2001). Après une post fixation de 24h des cerveaux dans du paraformaldéhyde, des coupes frontales de 40 µm de l'hippocampe sont réalisées sur un vibratome et

récupérées dans le tampon phosphate. On procède ensuite à une immunohistochimie sur les coupes flottantes (Malberg et al., 2001). Toutes les cellules BrDU-IR ont été comptées à l'objectif X100d'un microscope, dans la zone sous-granulaire et dans la couche granulaire de la partie rostrale du gyrus denté. Le traitement aux antidépresseurs n'a pas d'effet sur les contrôles, alors que les animaux SP montrent une augmentation de la prolifération cellulaire ($P<0.05$ vs S200098-10 mg/kg).

Le traitement aux antidépresseurs n'a pas eu d'effets sur les contrôles, alors qu'il a augmenté significativement la prolifération cellulaire chez les animaux SP à la dose de 10mg/kg ($P<0.05$ vs VEH) à des niveaux similaires au groupe contrôle.

Paradigme d'étude de la prolifération et de la différenciation





Nos études montrent que les rats SP répondent de façon positive à trois antidépresseurs différents, indiquant ainsi que le rat SP comme modèle de dépression a une "predictive validity".

ENVIRONMENTAL ENRICHMENT DURING ADOLESCENCE REVERSES THE EFFECTS OF PRENATAL STRESS ON ANXIETY-RELATED BEHAVIOURS AND STRESS REACTIVITY IN RATS

Morley-FletcherS., Rea M., Maccari S., Laviola G.

(soumis à European Journal of Neuroscience)

Dans cette dernière étude, nous posons la question de la réversibilité des altérations induites par le SP grâce à un environnement enrichi. Les animaux sont évalués lors de l'adolescence, un stade très plastique de développement plus sensible que les autres stades aux manipulations environnementales (voir Spear, 2000). Les rats mâles adolescents sont par paires de même sexe et appartenant à la même portée par cage, du sevrage jusqu'à ce qu'ils soient âgés de 60 jours, soit dans un environnement standard; soit dans un environnement enrichi. Les effets de l'environnement ont été évalués au niveau du comportement de jeu, du comportement émotionnel dans le labyrinthe en croix surélevé et de la réponse de l'axe HHS à un stress aigu. Tous ces paramètres sont altérés chez le rat SP.

- Les rats adolescents ont montré une diminution du comportement de jeu, une augmentation de l'anxiété dans le labyrinthe en croix surélevé et une prolongation de la sécrétion de corticostérone en réponse à un stress de contention.
- Après l'environnement enrichi, les rats SP ont montré une augmentation marquée du comportement de jeu, une réduction de l'anxiété et une régulation altérée de l'axe HHS avec un pic diminué et un retour aux niveaux de base similaire à celui des contrôles.

Ces résultats indiquent que la modification de l'environnement lors de l'adolescence peut constituer une stratégie efficace dans le traitement des altérations à long terme induites par un stress maternel.

Submitted to Brain Research

High corticosterone levels in prenatally stressed rats predict immobility behavior in the forced swim test. Effects of a chronic treatment with tianeptine.

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Abstract

Prenatally-stressed (PS) rats are characterized by a general impairment of the HPA axis indicating that this model has face validity with clinical features observed in a great majority of depressed patients. The prolonged corticosterone secretion shown by PS rats in response to stress was positively correlated with an increased immobility behavior in the forced swim test. To investigate the predictive validity of this model, animals were chronically treated with the antidepressant tianeptine (10 mg/kg i.p. for 21 days) following which a significant reduction of immobility behavior was observed. These findings suggest that the PS rat is an interesting animal model for the evaluation of antidepressant treatments.

Theme: Neural basis of behavior

Topic: Stress

Key Words: stress in utero; animal model; corticosterone; forced swim test; depression; tianeptine .

In human beings, increasing attention has been shown to the fact that stress exposure in early life predispose individuals to the development of affective and anxiety disorders in adulthood [15,16,33,40]. Depression, one of the most common human diseases, is characterized by a general lack of motivation and anhedonia leading to profound mood disturbances. One core biological feature of this disorder is the impairment of the hypothalamic-pituitary-adrenal (HPA) axis activity, at least in a subpopulation of depressed patients [18], together with high corticotropin releasing hormone (CRH) levels in the cerebrospinal fluid [29] and cholinergic hyperactivity [19]. Alterations in the sleep-wake cycle [22] and in a variety of circadian rhythms including cortisol rhythm [37], constitute also one of the hallmarks of depressive illness.

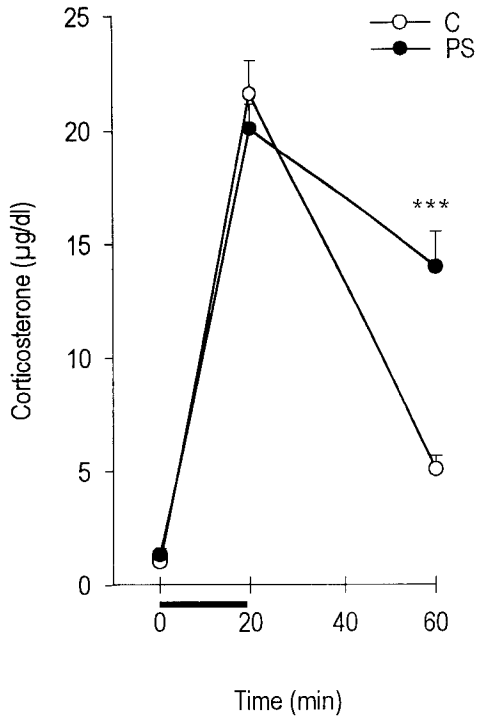
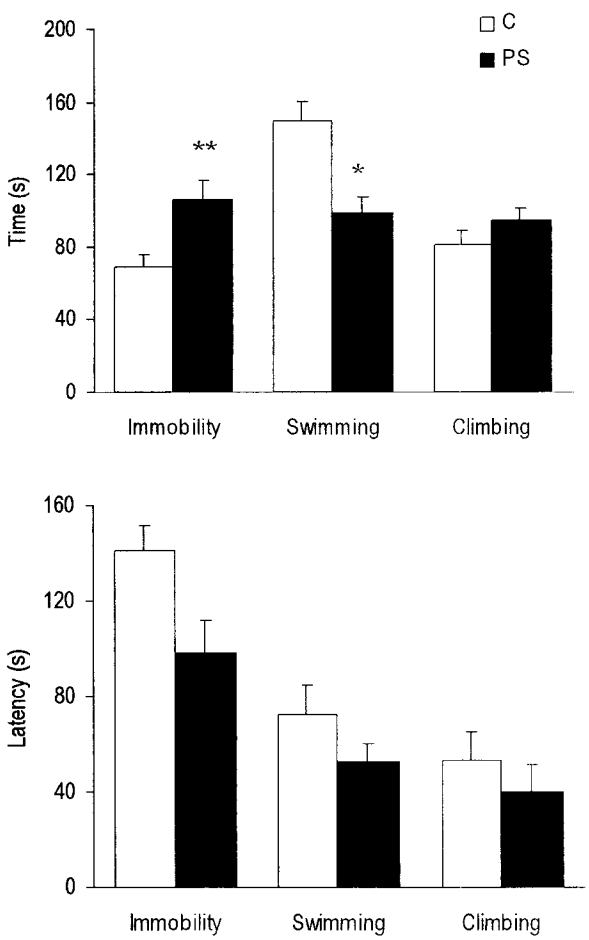


Fig. 1. Corticosterone secretion induced by 20 min restraint stress in control (C) and PS male rats. ***P <0.001 Results are expressed as mean ± SEM.



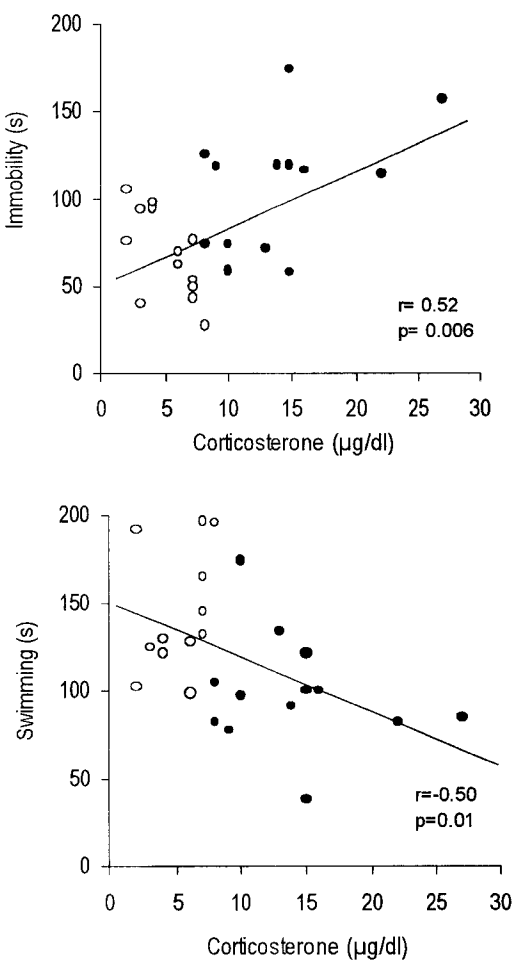


Fig. 2. *Left*: Effects of prenatal stress in the forced swim test. Immobility, Swimming and Climbing shown by control (C) and PS male rats at four months of age. *P <0.05 and ** P <0.01 vs controls. Results are expressed as mean ± SEM. *Right*: Pearson's correlation analysis between corticosterone levels (60 min after stress) and duration of immobility and swimming in the forced swimming test.

In rats, we have previously shown that prenatal stress (PS) can exert a number of alterations that are stable throughout life span, as they can be observed at early [17] as well as at later stages of development [36]. Many of these abnormalities parallel those found in human depressed patients, suggesting that PS rats could have high face validity for an animal model of depression. PS rats present impairment in the feedback inhibition of HPA axis activity [4,14,35], display acetylcholine hypersensitivity to stress and CRH challenge [9], and exhibit a phase shift in circadian activity of corticosterone secretion [20,21]. Finally, we have shown that PS has persistent effects on sleep-wake parameters inducing increased amounts and increased number of episodes of paradoxical sleep at adulthood [12]. More importantly, there is evidence of established correlation between HPA axis disturbances and sleep and behavioural abnormalities in PS rats [12,35]. In the present study, to further validate the PS rat as an animal model for depression, we investigated possible correlation between corticosterone response and behavior in the forced swim test, classically used to evaluate antidepressant efficacy [30]. Then, a chronic treatment with the antidepressant tianeptine, known to act on the HPA axis [10,38] was conducted to assess normalization of the behavioral response.

Adult virgin Sprague-Dawley female rats (Iffa Credo, France) were individually housed in the presence of a sexually-experienced male for a whole estrous cycle. Pregnant females were then individually housed in plastic cages and randomly assigned to Prenatal Stress (PS) or Control (C) group. For all experiments, animals were allowed *ad libitum* access to food and water, and maintained on a regular light/dark cycle (lights-on 07:00 to 19:00 h) with constant temperature (23°C) and humidity (60%). Prenatal stress was conducted as previously described [4,24].

After weaning, male rats from each experimental group were collectively housed. At 3 months of age, C and PS male rats (n=13 per group) were tested for plasma corticosterone response to a 20-min restraint stress in the morning. Rats were moved to an adjacent room and individually placed in a restraint transparent tube (d=7 cm; l=19 cm). Blood samples were collected via the tail vein in heparinized tubes as previously described [4,23]. Plasma corticosterone levels were measured using a RIA Kit (ICN Biomedicals) with a highly specific corticosterone antibody and a detection threshold of 0.1 µg/100 ml. The intra- and inter-assay coefficients of variation were 5 and 11 % respectively.

After 3 weeks, rats were assessed in the forced swim test. An adapted version of the Porsolt test was used. On pre-test day animals were individually plunged for 15 min into a glass cylinder (d= 25 cm; h= 59 cm) containing 36 cm of water maintained at 25°C. They were then removed from the water and allowed to dry in a heated room before returning to their home cages. 24 hours later (test day), rats were put back into the cylinder for 5 min. Both latency and duration of the three following behaviors were measured: immobility (floating in the water with only movements necessary to keep the head above water); swimming (active swimming motions around cylinder); climbing (active movements with forepaws usually directed towards the walls).

In a second set of animals, C and PS male rats (n=7 for each treatment group) were injected daily for 21 days either with saline or with 10 mg/kg tianeptine (Institut de Recherches Internationales SERVIER, IRIS France). Animals underwent the forced swim procedure on the last two days of chronic treatment.

All experiments were conducted in accordance with the principles of laboratory animal care (NIH publication No. 85-23, revised 1985 or European Communities Council Directive of 1986, 86/609/EEC).

For the first experiment, ANOVA with two levels of group (control vs prenatal stress) and three levels of time (basal, peak, return to basal level), was used to compare values of corticosterone levels, whereas unpaired Student's t-test was used to analyze data of the forced swim test. Relationships between behavior and stress-induced corticosterone levels were assessed by Pearson's correlation analysis. For the second experiment, ANOVA with two levels of group and two levels of treatment (saline vs tianeptine) was used followed by Student's t-test when appropriate. Significance level was set at P <0.05.

Fig. 1 shows that PS induced, as expected, increased stress-induced corticosterone levels (ANOVA, group by time interaction, $F_{2,48}$ =19.31 P <0.001). Specifically, prolonged corticosterone values were observed in PS rats 60 min after the initiation of stress procedure (t-test: t =5.40, P <0.001).

Fig 2 shows that in the forced swim test PS increased amount of immobility (t-test: t =3.04 P <0.01) whereas it reduced duration of swimming behavior (t-test, t =3.62 P <0.001). Climbing behavior was not affected. Also latency to immobility was significantly reduced in PS rats with respect to C group (t-test: t =2.40, P <0.05).

A significant correlation between plasma corticosterone levels and the behavioural scores in this test was observed (see fig. 2 right panel). Namely, corticosterone levels measured 60 min after stress were positively correlated with duration of immobility (r = 0.52, P < 0.01) and negatively with duration of swimming (r = -0.50, P < 0.01).

Fig. 3 shows that chronic treatment with tianeptine significantly affected immobility behavior in the forced swim test (ANOVA group by treatment interaction $F_{1,24}$ =16.33, P <0.001, and $F_{1,24}$=3.96, P <0.05 for duration and latency respectively). As expected, PS-VEH animals showed increased duration of immobility (t-test: t =2.38, P <0.05) and reduced latency (t =2.09, P <0.05) with respect to the corresponding control group. Tianeptine markedly decreased time spent in immobility in PS rats (t-test: t=2.34, P <0.05) whereas latency was

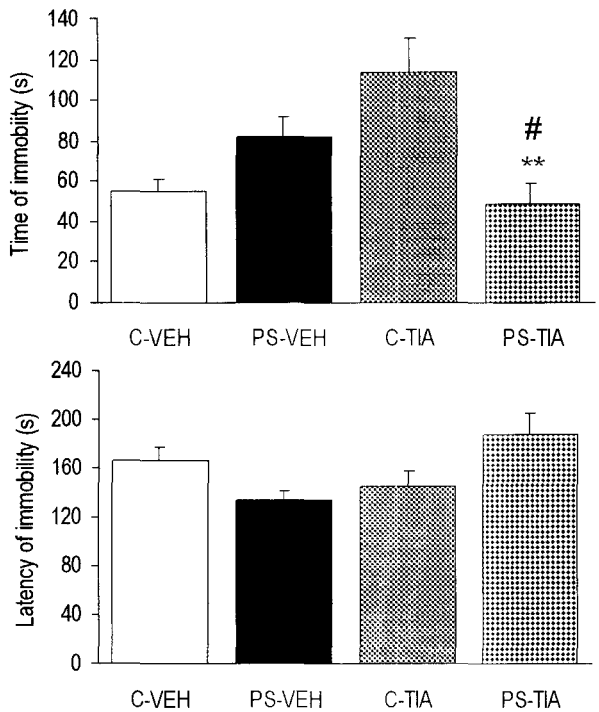


Fig. 3. Influence of chronic tianeptine treatment on immobility and latency in the forced swimming *test.* Control (C) and PS rats were injected for 21 days either with saline (VEH groups), either with 10 mg/kg tianeptine (TIA groups). **P* <0.05 and ***P* <0.01 PS vs controls. #P<0.05 PS-VEH vs PS-TIA. Results are expressed as mean ± SEM.

Table 1. Effects of chronic tianeptine treatment on swimming and climbing in the forced swim test. Prenatal stress significantly decreased duration of the swimming behavior. This profile was reverted following chronic tianeptine treatment. No differences were observed for climbing behavior between the groups. **P* <0.05 vs C-VEH; $*P* <0.05 vs PS-VEH; #*P* <0.05 vs C-TIA.

	C-VEH	PS-VEH	C-TIA	PS-TIA
Swimming (s)	151.7 ± 13.7	114.3± 13.4	108.1± 13.4*	168.4± 10.1 $#
Climbing (s)	93.3± 9.6	103.4 ± 11.2	77.0± 8.2	82.6± 13.2

significantly increased (t-test: t =2.74, P <0.05). In the control group, antidepressant treatment increased immobility (t-test: t =3.30, P<0.01) and had no effect on latency.

When considering swimming and climbing activities (see table 1), ANOVA revealed a significant group by treatment interaction for duration of swimming ($F_{1,24}$ = 15.27, P <0.001). Namely, PS-VEH showed a trend for reduced levels of swimming (t-test: t =2.02, P =0.06) when compared with the corresponding control group. Chronic treatment with tianeptine increased levels of swimming in PS rats (t-test: t =3.38, P <0.05 vs PS-VEH and t =3.59, P <0.05 vs C-TIA) whereas it decreased duration of swimming in controls (t-test: t =2.27, P <0.05 vs C-VEH). Climbing behavior was not affected by antidepressant treatment.

In the present study, PS male rats previously characterized by their prolonged corticosterone response to an acute stress, displayed high levels of immobility behavior in the forced swim test. Our findings confirm and extend previous data obtained in female rats in other PS models [1,11]. Behavioral performance in the forced swim test at 4 month and corticosterone response at 3 month of age were strongly correlated. Moreover, there is strong evidence for the involvement of corticosterone secretion in the behavioral performance in the forced swim test [3,28]. In this view, the finding of such a correlation reinforces our previous data indicating a prominent role of HPA axis in behavioral alterations [12,35]. In addition to glucocorticoids, other factors could be involved in the effect of PS on immobility. For instance, the increased amount of REM sleep shown by PS rats [12,31] could be also taken into account, given that REM sleep deprivation, a non-pharmacological intervention known to exert antidepressant effects, induces anti-immobility effect in the forced swim test [2].

Since antidepressant must be administered for at least two weeks before clinical benefits are seen in human beings, PS rats were administered tianeptine chronically. Chronic antidepressant treatment significantly reduced their immobility behavior, thus indicating predictive validity for this model. Given that tianeptine has shown to attenuate HPA axis activation in stressed animals [10,18,34], its restoring action on the hyperactivity of the HPA axis of PS rats has to be hypothesized. Suppression of behavioral deficits by tianeptine has already been reported in other studies [5,7], and its ability to reduce immobility in the forced swim test supports the clinical aspects of its antidepressant action [38]. Tianeptine increased immobility in control rats. Effects of chronic tianeptine in non-stressed animals have been reported also in other studies with respect to memory performance and HPA axis activity [7,10].

Besides the already reported activity on the HPA axis, tianeptine has shown to prevent stress-induced deficits in neuroplasticity [8,25,27,39]. In stress-related neuropsychiatric disorders such as recurrent depressive illness, there is evidence of structural changes in the hippocampus, a brain region extensively studied with regard to stress and antidepressants' action [32] and a recent hypothesis links the reversal or prevention of these structural changes to the antidepressant therapy [13,26]. Little is known about effects of exposure to stress during a critical period such as prenatal life, but there is recent evidence of a lifespan reduction of neurogenesis in the hippocampal region [23]. Since tianeptine increases neurogenesis in adult animals [8], its antidepressant effect in PS rats could be related to its regulatory properties in synaptic plasticity.

Although the face validity and predictive validity of the PS rat model requires certainly further validation, the present data show that it could represent an interesting animal model for the testing of antidepressants' efficacy. Since antidepressants are commonly given to patients presenting affective disorders, our results indicate also that the use of stressed rather than normal animals might nevertheless provide a more useful model in evaluating the mechanism of their action.

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En préparation

Chronic treatment with imipramine affects behavior, hippocampal corticosteroids and cortical 5-HT_{1A} receptor expression in prenatally stressed rats

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Strong evidences suggest that exposure to stress experience in early life can exert long-lasting perturbations of an organism's physiological homeostasis. In the rat, many of the abnormalities found in prenatally stressed (PS) animals, such as impairment of the feedback inhibition of the hypothalamus-pituitary adrenal (HPA) axis, disturbances in a variety of circadian rhythms and increased anxiety, parallel those found in human depressed patients. Although the PS rat has been quite well characterized for its face validity as an animal model for some biological aspects of depression, little is known about its predictive validity. In the present study, PS rats were treated chronically with the tricyclic antidepressant imipramine (10 mg/kg i.p. for 21 days) and assessed in tests of anxiety and in the forced swim test. Levels of 5-HT_{1A} receptor mRNA in the cortex were measured, as well as changes in binding to corticosteroid receptors in the hippocampus. PS rats were characterized by high levels of self-grooming behavior when faced with a conspecific and reduced exploration of the open arms in the elevated plus-maze, together with an increased immobility in the forced swim test. Imipramine reduced immobility in the forced swim test, increased binding of hippocampal corticosteroid and decreased levels of cortical 5-HT_{1A} mRNA in PS rats. Normalization of HPA axis and 5-HT system by imipramine is consistent with efficacy of pharmacotherapy with antidepressants in human beings. These results indicate that PS rats could be a suitable animal model for the evaluation of antidepressant efficacy.

Key words: prenatal stress; imipramine; 5-HT_{1A}mRNA; forced swim test; anxiety; animal model

Depressive disorders are among the most common human diseases in that approximately 11% of all adult human beings experience a time period of depression at least once in their lives (Judd, 1995). Impairment of the hypothalamic pituitary adrenal (HPA) axis is one of the core biological features of depression, at least in a subpopulation of depressed subjects (Holsboer et al., 1984; Rubin et al., 1987), and dysfunctions in the serotonergic system have also been reported (Meltzer and Lowy, 1987). Disrupted sleep patterns, with increased amount and decreased latency of paradoxical sleep are observed (Kupfer and Reynolds, 1992), together with a phase shift in circadian activity rhythms (Rosenwasser and Wirz-Justice, 1997). There is also a wealth of evidence of comorbidity of depression with anxiety disorders (Stahl, 1993; Rouillon, 1999).

In humans, exposure to stressful conditions during a critical period such as perinatal life has been reported to increase vulnerability to develop

behavioral and affective disturbances in adulthood (Glover, 1997; for a review see Heim and Nemeroff, 2001; Weinstock, 2001). In this regard, stressful life events are among the most potent environmental factors that may trigger or induce depressive episodes (Sapolsky, 1996; Kessler, 1997). The stress concept of mood disorders has led to the hypothesis that stress-induced models of depression have good construct validity (for a review see Willner, 1997; Willner and Mitchell, 2002). Among the different animal models developed, the prenatal stress (PS) model in the rat has high construct and face validity.

Indeed, in PS rats a long-lasting impairment of HPA axis feedback inhibition has been reported (Koehl, et al. 1997; 1999; Vallee et al., 1997), and there is evidence of dysfunctions at the level of the serotonergic system (Peters 1986). Alterations in sleep-wake parameters with increased amounts of paradoxical sleep (Dugovic, et al. 1999) are observed, as well as disturbances in a variety of circadian rhythms such as corticosterone secretion and locomotor activity (Koehl et al., 1997; 1999; Van Reeth et al., 1998). Finally, PS rats show a general impairment at the behavioral level including reduced sexual behavior (Ward, 1983) and increased reactivity and anxiety when faced with novelty (Vallee, et al. 1997; Weinstock, 2001). Moreover, in this model the alterations reported are stable throughout life-span, since they can be observed at early (Henry et al., 1994) as well as later stages of development (Vallee et al., 1999). This aspect makes a dramatic difference with respect to the transitory stress-induced disturbances observed in other animal models (see Yadid et al., 2000).

Very little is known about the predictive validity of this model with classical antidepressants (see Alonso et al., 1999). To this purpose, adult PS male rats were chronically treated (three weeks) with the tricyclic antidepressant imipramine. In order to evaluate the efficacy of the antidepressant in reversing the anxiety behavior, animals were assessed for their response to a novel conspecific in the social interaction test (File, 1980) and to a novel environment in the elevated plus maze test. At the end of the third week of treatment, rats were observed in the forced swim test, a test classically used to validate antidepressant activity (Porsolt, 1978). To assess normalization of the HPA axis and the serotonergic system by chronic imipramine treatment, we measured corticosteroid receptor binding in the hippocampus, as well as levels of mRNAs encoding 5-HT_{1A} receptors in the frontal cortex.

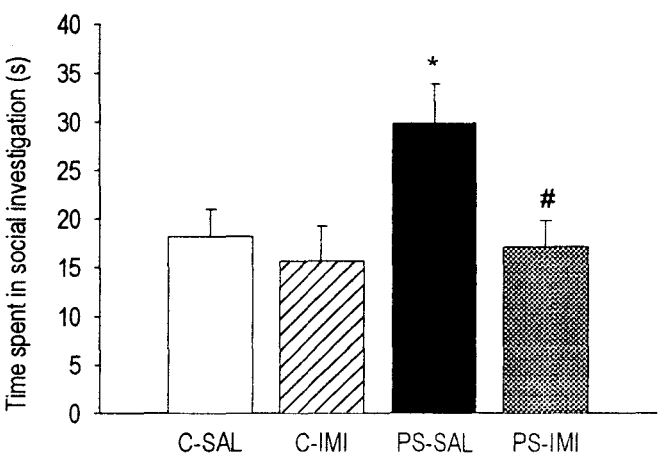
MATERIAL AND METHODS

Animals. Sprague-Dawley nulliparous female rats weighing approximately 250 g, were purchased from a commercial breeder (Iffa Credo, France). Animals were kept at constant temperature (22±2°C), with a regular light/dark cycle (lights-on at 7.00 p.m.). Water and food were available *ad libitum*. For a week after arrival animals were group-housed (4 per cage) to coordinate their estrous cycle. Females were then placed with a sexually experienced male for a week, after which they were housed individually in Plexiglas cages (50x35x25 cm). Pregnant females were then randomly assigned to Prenatal stressed (PS) or control group (n=12 for each group).

Stress procedure. Prenatal stress was conducted as previously described (Maccari, et al. 1995; Barbazanges et al., 1996): on day 14 of pregnancy until delivery pregnant female rats were individually placed in plastic transparent cylinders (diam.=7 cm; lenght=19 cm) and exposed to bright light for 45 min. Animals were submitted daily to three stress sessions starting at 09:00, 12:00 and 17:00 h, whereas control pregnant females were left undisturbed in their home cages. Male and female offspring were weaned 21 days after birth, and only male offspring from litters containing 10 - 14 pups with a comparable number of males and females was used in the experiments. A maximum of two male pups were taken from each litter to remove any 'litter effect' (Chapman and Stern, 1979). After weaning, male rats from each experimental group (control vs PS) were housed in groups of four and maintained in the same environmental conditions until experiments started. All experiments were conducted in accordance with the principles of laboratory animal care (NIH publication No. 85-23, revised 1985 or European Communities Council Directive of 1986, 86/609/EEC).

Antidepressant drug administration. Imipramine (Sigma) was dissolved in saline (0,9%) and intraperitoneally (i.p.) injected at the dose of 10 mg/2ml/kg. Injections were performed once daily for 21 days two hours before lights-off (18:00 h). Controls received injections of saline.

Social interaction test. Following a week of antidepressant treatment, animals were assessed in the social interaction test. Such a test measures the anxiety rats display towards a non familiar conspecific. Here the effects of chronic imipramine were investigated in conditions (low light, unfamiliar environment) which allow the detection of both anxiolytic and anxiogenic effects (File, 1980). Each experimental animal (n=12 for each group) was tested with a standard-untreated partner of approximately equal weight that had been previously isolated, and only the interaction initiated by the treated rat was scored. In order to arouse behavior of the standard rat before social confrontation and to increase aggressive interactions, such animal was previously placed for 25 min in the testing environment (square wooden arena,



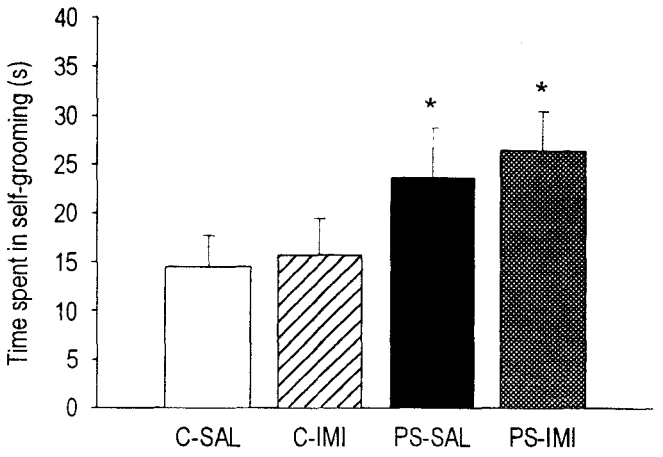
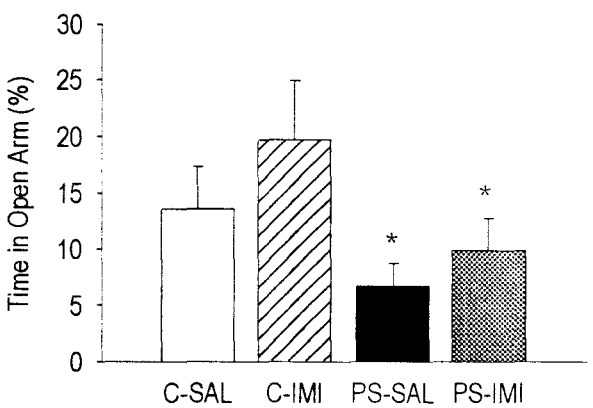


Fig. 1. Effect of chronic Imipramine treatment (IMI, 10 mg/kg i.p) in the social interaction test. Time (mean ± SEM) spent in social investigation (A) and self-grooming (B) shown by control and PS rats after one week of treatment (n=12 each treatment group). Prenatal stress increased both behaviors. * $p < 0.05$ vs controls. Chronic Imipramine treatment reduced social investigation in PS rats whereas it had no effect on grooming behavior. # $p < 0.05$ vs PS-SAL.



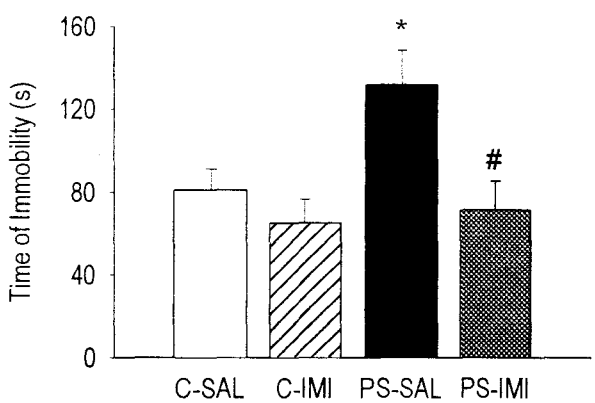


Fig. 2. Effect of chronic Imipramine treatment (IMI, 10 mg/kg i.p) in the elevated-plus maze test. Percentage of time spent in open arms (open/open + closed) measured in the elevated plus maze over 5 min test shown by control and PS rats after two weeks of treatment (n=12 each treatment group). Prenatal stress reduced exploration of the open arms. Chronic Imipramine had no effect on the profile of anxiety in PS rats. *$p < 0.05$ PS vs controls.

Fig. 3. Effect of chronic Imipramine treatment (IMI, 10 mg/kg i.p) in the forced swimming test. Time (mean ± SEM) spent in immobility of control and PS adult rats after three weeks of treatment (n= 10 each SAL group; n=12 each IMI group). Prenatal stress increased immobility time whereas Imipramine treatment reduced it. *$p < 0.05$ PS-SAL vs C-SAL; #$p < 0.05$ vs PS-SAL.

50x50x20 cm) with a female with whom it had been previously familiarized (adapted from Mormede et al., 1990). Following this period the female was taken and the experimental rat was introduced, and the time spent in active social interaction, exploration of the unfamiliar environment, and self-grooming during a 20 min session was video recorded. The first and fourth 5 min of each session were manually scored (Observer 20 Noldus, Wageningen, The Netherlands) by an observer blind to the rat treatment.

Elevated-Plus Maze. On the second week of treatment animals were assessed for the elevated-plus maze response in a 5 min session. The elevated-plus maze was wooden made, according to the specifications of Pellow and coworkers (1985). The apparatus consisted of two open arms (50x10 cm), alternating at right angles, with two arms enclosed by 40 cm high walls. The fours arms delimited a central area of 10 cm^2. The whole apparatus was placed 1m above the floor. A 3 cm high wooden rim prevented the rats from falling off the open arms.

The forced swimming test. The test was conducted at the end of the third week of treatment. We used an adapted version of the forced swim test (Porsolt, et al. 1978). A cylindrical container (height= 59 cm; diam.=25 cm) was filled with 25°C water up to a level of 36 cm. In the first session, rats were placed in the water for a 15 min pretest. Then, they were removed from the water and allowed to dry in a heated room before being returned to their home cages. 24 hours later (test day), rats were put back into the cylinder for 5 min. During this time, the duration of active escape behavior (swimming or climbing) and passive behavior (immobility, the rat is completely still except for isolated movements to keep the head above the water) was manually scored (Observer 20, Noldus Technology, Wageningen, The Netherlands) by an observer blind to the rat treatment.

Following behavioral observations animals were sacrificed by decapitation, brains were quickly removed, cerebral cortex and hippocampus dissected out, frozen on dry ice and stored at –80°C until neurochemical analysis.

Hippocampal corticosteroid receptor binding. In order to eliminate endogenous corticosterone, an exchange assay was used for both type I and type II corticosteroid receptors as previously described (Casolini, et al. 1993; 1997; Henry et al. 1994; Maccari et al. 1995; Koehl et al., 1999) with some modifications. In particular, total corticosteroid receptors were estimated by a single saturating ligand concentration, maximal individual binding (for a previous use of this methodology see Catalani et al., 2002). Aliquots of cytosol (140 µl) were incubated with tritated corticosterone (specific activity 76.5 Ci/mmol; New England Nuclear, Italy) at a concentration of 40 nM. No specific binding for tritated corticosterone was determined in the presence of a 500-fold excess of unlabeled corticosterone. Since previous works had shown that affinity of corticosteroid receptors is not affected by exposure to prenatal stress (Henry et al., 1994; Barbazanges et al., 1996; Maccari et al., 1995; Koehl et al., 1997; 1999) in the present study we just measured density of corticosteroid receptors.

Quantitative determination of 5-HT_{1A} receptor mRNA in the cortex. The method used to measure mRNAs was based on a competitive reverse transcriptase-polymerase chain reaction (RT-PCR) technique (Siebert and Larrick 1992), in which mRNAs of analyzed gene are reverse-transcribed and amplified in the presence of a homologous deleted internal standard mRNA using a RT-PCR Access System Kit (Promega Madison, WI, USA). Quantitative determination of 5-HT_{1A} receptor mRNA in the frontal cortex was performed as described by Le Poul and coworkers (2000). Reverse transcription (45 min at 48°C) proceeded with 0.5 µg of total tissue RNA in the presence of standard deleted RNA at increasing dilutions (10^{-6} to $3x10^{-8}$). The sequences of the upstream and down-stream oligonucleotide primers (Albert et al., 1990) were 5`-CTCTACGGGCGCATCTTCAGA-3` (nucleotides 762-782), and 5`-CCCAGAGTCTTCACCGTCTTC-3` (nucleotides 1165-1145). PCR amplification was performed with 1-2 units of Tfl DNA polymerase, 1 m M MgSO4 and 1 pg/µl of each primer for 30 cycles (1 min at 95°C, 1 min at 58°C and 1 min at 72°C). After electrophoretic separation in 2% agarose gel stained with 4% ethidium bromide, both standard and tissue RT-PCR products were quantified with a gel analyzer software (NIH 1.6).

Statistics. Data from the behavioral tests and from the 5-HT_{1A} RT-PCR measurement were analyzed using parametric analysis of variance (ANOVA) with two levels of group (Control vs PS) and two levels of treatment (saline vs imipramine) as between subject variables. Planned comparisons with contrast analysis followed when appropriate. Because data from the corticosteroid receptor binding assay did not meet strict normality criteria, the Mann-Whitney rank sum test was used in this case. Significance was set at $p < 0.05$.

RESULTS

Effects of imipramine on social interaction test

PS rats showed (see Fig. 1) higher amount of anogenital sniffing (group effect, $F_{(1,44)} = 4.06$; $p < 0.05$) and higher levels of self-grooming behavior (group effect, $F_{(1,44)} = 5.98$; $p < 0.05$) with respect to control animals. Overall, no differences were

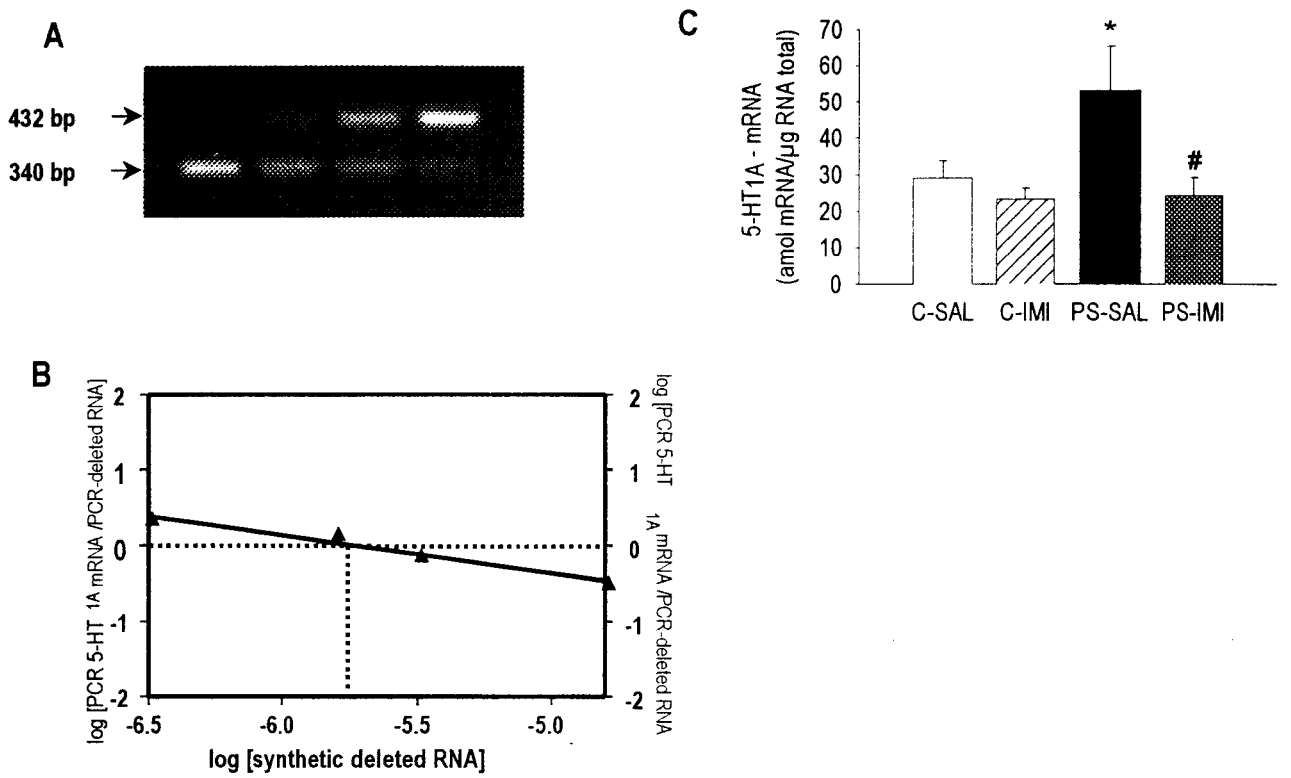


Fig. 4. Quantitative RT-PCR of 5-HT_{1A}–mRNA in the cortex.
A: Example of electrophoretic separation in a 2% agarose gel stained with ethidium bromide of RT-PCR products. A, B, C, D represent each dilution of synthetic deleted mRNA whose initial concentration was 0.72 µg/µl (A:10^{-5}; B:3.10^{-5}; C:10^{-6}; D:3.10^{-7})
B: Plot for the quantification of PCR products : the logarithmic ratio of the amounts (OD measurements) of the specific mRNA (432 bp) over those of the synthetic deleted RNA (340 bp) is plotted as a function of the logarithm of each serial dilution of the synthetic deleted RNA. The intersection of line with the x-axis gives the equivalent dilution of the synthetic deleted RNA, thus the equivalent amount of specific 5-HT_{1A} mRNA in the rat.
C: Effect of chronic Imipramine treatment (IMI, 10 mg/kg i.p) in control and PS group (n=8-9 for each SAL group; n=5 for IMI groups). Values are expressed as atomole-mRNA per µg of total RNA. Prenatal stress increased 5-HT_{1A}–mRNA expression whereas Imipramine treatment reduced it. *$p < 0.05$ vs C-SAL; #$p < 0.05$ vs PS-SAL.

Table 1. Effect of chronic Imipramine treatment (IMI, 10 mg/kg i.p) on binding capacities of corticosteroid hippocampal receptors in control and PS adult rats (n=6 for each treatment group). Chronic treatment with the antidepressant increased Bmax in PS rats. #$p < 0.05$ vs PS-SAL.

Group	Treatment	Bmax (fmol/mg.prot.)	Bmax % of controls
Controls	Saline	503.2 (57.3)	100
Controls	IMI	439.6 (64.9)	88
PS	Saline	405.7 (17.7)	80
PS	IMI	495.9 (40.0) #	98

observed (data not shown) between PS and control group for other social behavioral items (following the partner, crawling over, boxing and wrestling) or for the time spent in exploring the environment (non-social behavior: activity in the cage, rearings). Imipramine treatment significantly reduced in PS rats the time spent in anogenital investigation ($F_{(1,44)}$ = 5.37; p <0.05), whereas it had no effect on grooming behavior neither on exploratory activity (223.8 s ± 8.7 for saline-treated vs 216.7 s ± 8.7 for imipramine-treated PS group). As a whole, no effects of chronic antidepressant treatment were observed for control group.

Effects of imipramine on the elevated plus-maze

PS rats spent a reduced percentage of time in the open arms (group effect, $F_{(1,44)}$ = 5.20; p <0.05) when compared to controls (Fig. 2). The same profile (data not shown) was observed for the number of entries into the open arms ($F_{(1,44)}$ =5.02; p < 0.05). There were no differences between the different groups for locomotor activity (closed arm entries, data not shown). No effects of chronic imipramine treatment were observed (treatment effect, ($F_{(1,44)}$= 0.62 n.s.).

Effects of imipramine on forced swim test

PS rats spent more time being immobile (fig. 3) than control animals (group effect, $F_{(1,40)}$ = 4.56; p < 0.05). Chronic imipramine treatment significantly reduced immobility time in PS rats ($F_{(1,40)}$ = 8.04; p < 0.01) whereas it had no effect on control group ($F_{(1,40)}$ = 0.70 n.s).

Moreover, no real differences between non-treated PS and control groups were observed either for climbing or for swimming activities. In the contrary, imipramine treatment increased levels of climbing ($F_{(1,40)}$= 10.08; p <0.01) in PS rats whereas it had no effect on control group (data not shown).

Effects of imipramine on hippocampal corticosteroid receptor density.

In the hippocampus (table 1), a trend for reduced levels of receptor binding (-20% as compared to control-non treated group) was found for PS animals. Following imipramine treatment receptor binding was significantly increased in PS rats (Mann-Whitney, U=5; p < 0.05). No effects of the antidepressant were observed for control groups.

Effects of imipramine on cortical 5-HT_{1A} receptor expression

In the frontal cortex (fig. 4), levels of 5-HT_{1A} receptor mRNA were significantly higher (+81% as compared to control-non treated group) in PS rats than in controls (group effect, $F_{(1,23)}$ = 4.28 p < 0.05) and were markedly reduced (-54% as compared to PS group) to levels equal to controls following antidepressant treatment ($F_{(1,23)}$ = 4.74 p < 0.05). No significant effects of imipramine were observed for the control group (- 22% as compared to saline treated animals).

DISCUSSION

In human beings, the clinical effects of antidepressant drugs appear no earlier than two or three weeks after the onset of treatment. However, it has to be noted that a limited number of studies have employed chronic administration of antidepressants during a clinically relevant time in animals (Monleon, 1995; Reul et al., 1993; Yau et al., 2002b). Moreover, the study of the efficacy of pharmacotherapeutic intervention in psychiatric disorders has often been addressed to non-stressed animals, whereas, clinically, antidepressants do not affect the mood nor the behavior of non-depressed individuals. Therefore, the interpretation of results from normal rats has a limited relevance for understanding the neurochemical abnormalities observed in depression and the beneficial effects of antidepressant drugs. This is why, in the present study, we investigated the predictive validity of the PS rat as an animal model of depression in treating animals chronically for three weeks with the tricyclic antidepressant imipramine. Our results indicate that PS rats showed reduced immobility behavior in the forced swim test, normalization of the HPA axis and serotonergic system, whereas their behavioral response in anxiety tests was slightly affected. Antidepressant treatment had no effect what so ever on control animals.

Behavioral performance of PS rats and effects of chronic imipramine treatment

PS rats showed higher immobility behavior in the forced swim test. Chronic imipramine significantly reduced immobility behavior in PS rats whereas it had no effect in controls. Our results are in accordance with a previous work of Alonso and coworkers (1991; 1999) who showed reduced immobility following chronic antidepressant treatment in prenatally stressed females. Because the immobility behavior in the forced swim test is reduced by imipramine one can propose that PS rats may be more prone to adopt passive strategies in response to inescapable stressful situations, i.e., a behavior that might be considered, to some extent, as a reflection of a depressive-like state (Porsolt, 1978).

In our study, PS rats showed a higher amount of self-directed behavior together with a peculiar increase in anogenital investigation of the partner in the social test, and they showed reduced exploratory

behavior of the open arms in the elevated plus maze. These results confirms and extend those previously reported indicating enhanced reactivity and emotionality in these animals (Vallee et al., 1997; Weinstock, 2001). Moreover, in other studies self-grooming behavior has been associated with activation of the HPA axis (Dunn and File, 1987; van Erp et al., 1994). This behavior is often related to de-arousal following stressors given that, following exposure to non-aggressive conspecific, it first increases and then decreases (Spruijt et al., 1992). The higher levels of self-grooming behavior observed throughout the session in PS rats indicate an increased level of arousal which is consistent with the sustained activation of the HPA axis already reported for these animals when faced with novelty (Henry et al., 1994; Maccari et al., 1995). In the contrary, the increased amount of social investigation shown by PS rats could appear in contrast with the self-grooming profile, since high levels of self-directed behavior are normally associated with a concomitant reduction of social behaviors. Moreover, social interactions often decrease when animals are faced with an alien partner in a novel environment (File, 1988). Decreased social interaction between rats placed in a novel environment is then considered to be an adaptive response to an anxiogenic situation. Although this hypothesis requires further investigation, the increased investigation behavior observed in PS rats associated with higher levels of self-grooming could be interpreted as a different coping strategy (Weinstock, 1997), or more specifically, a maladaptive response toward a mild environmental challenge. Interestingly, PS rats showed a selective reduction of investigation behavior but no changes in grooming nor elevated plus maze response following antidepressant treatment. Since decreases in anxiety in an unfamiliar test arena are reflected in decreased social interaction, an anxyolitic effect of imipramine on this specific pattern could be hypothesized (File, 1980). The absence of anxyolitic effects of imipramine in the elevated plus maze could be related to a too short length of exposure to antidepressant treatment to exert its effects (Kurt et al., 2000). In fact, much longer treatment duration have been reported to be effective in reducing anxiety behavior in this test (see Yau et al., 2002b).

Normalization of HPA axis the serotonin system in PS rats by imipramine

In the present study, prenatal stress induced reduced binding capacity for hippocampal corticosteroid receptors, and upregulation of cortical 5-HT_{1A} gene expression at the mRNA level. These results confirm and extend previous data showing downregulation of corticosteroid receptors in PS rats (Maccari et al. 1995; Barbazanges et al., 1996), as well as impairment of the serotonergic system. For this latter, increased 5-HT_2 receptor density in the cerebral cortex and decreased density of the same receptors in the hippocampus has been reported as a consequence of PS (Peters 1988; 1990).

There is a reciprocal influences between the serotonin system and the HPA axis (de Kloet, et al. 1986; Meijer and de Kloet, 1994; Lanfumey et al., 2000). Most importantly, dysregulation of the HPA axis and malfunction of the serotonergic system have been implicated in the pathophysiology of depression and anxiety (Meltzer and Lowy, 1987). Among the 5-HT receptors, 5-HT_{1A} subtype are excellent candidates to mediate functional abnormalities in depression (Lesch, 1991; Lanfumey et al., 2000). These receptors are highly expressed in the cortex and in the hippocampus (Lanfumey and Hamon 2000), and higher levels of 5-HT_{1A} receptors have been reported in the prefrontal cortex of nonviolent depressed suicides than in postmortem brains (Matsubara et al., 1991). In our study, corticosteroid receptor density was increased following imipramine treatment, whereas the expression of 5-HT_{1A} receptors at the mRNA level was downregulated. Such a profile is consistent with the well reported efficacy of antidepressants to inhibit some changes evoked by glucocorticoids as well as hyperactivity of HPA axis often observed in depression (Seckl and Fink 1992; Reul et al. 1993; Holsboer and Barden 1996; Yau et al., 2002a). The normal responsiveness of corticosteroid receptors can be restored by chronic treatment with antidepressant drugs that may act through the serotonin system (Barden et al., 1995). In this regard, chronic treatment with tricyclic antidepressants results in downregulation of cortical 5-HT_{1A} receptors suggesting the specific involvement of cortical 5-HT_{1A} receptors in the mechanism of action of this type of antidepressants (Srinivas, et al. 2001). Interestingly, in control rats, levels of corticosteroid receptors and 5-HT_{1A} receptor mRNA were not affected by the same antidepressant treatment. This profile is consistent with other studies on the effects of chronic antidepressant treatment in non-stressed rats (Hrdina 1987; Caccia et al., 1993; Dewar et al., 1993).

Interestingly, the cortical 5-HT_{1A} receptors could be the main site for the behavioral alterations observed in PS rats in the present study. Indeed, the increased immobility in the forced swim test and the general profile of anxiety may be consistent with the increased amount of cortical 5-HT_{1A} expression. For example, studies conducted on 5-HT_{1A} knockout mice have reported a reduced immobility behavior in the forced swim test (Ramboz et al. 1998). Also, in the Flinder Sensitive Line (Overstreet et al., 1996), there is evidence of higher density of postsynaptic 5-HT_{1A}

receptors in the frontal cortex, with low scores of social behavior and greater immobility in the forced swim test (Gonzalez et al., 1998). Our findings of a reduced immobility observed in PS rats following imipramine treatment might reflect the observed downregulation of 5-HT_{1A} expression postulated as a mechanism for antidepressant action (Blier and de Montigny 1994). Conversely, the absence of effects of imipramine treatment on control animals for this parameter could be related to the above reported profile of antidepressant action on 5-HT_{1A} receptors in non-stressed rats (Dewar et al., 1993). Anxiety is usually associated with increase endogenous serotonin, whereas anxiolysis tends to be associated with its decreased endogenous levels (Clement and Chapouthier, 1998). The 5-HT_{1A} receptor subtype plays an important role in the modulation of anxiety. In fact, some experimental data suggest that anxiolytic potency of 5-HT_{1A} receptors may be associated with presynaptic sites such as in the raphe nuclei (Jolas et al., 1995), whereas anxiogenic effects could be modulated by postsynaptic receptors such as in the cortex, hippocampus or amygdala (Andrews et al., 1994).

Although additional work is necessary to understand the role of serotonergic system and stress in the regulation of behavior, the differential patterns of adaptation in the various behavioral tasks following chronic imipramine treatment could reflect the possibility that the tasks are subserved by 5-HT_{1A} receptors located in different brain regions, and that these 5-HT_{1A} receptors differentially adapt to chronic imipramine treatment. This hypothesis is supported by a study of Gonzales and colleagues (1996) who have reported that 5-HT_{1A} receptors in the amygdala modulate anxiety in the social interaction test but not in the elevated plus maze.

Conclusions

In the present study, chronic imipramine treatment was effective in restoring some of the abnormalities observed in PS rats at the behavioral and neurochemical levels, thus providing evidence of the validity of this model for the study of antidepressants' action. Although it is evident that animal models of depression represent an oversimplification of a highly complex illness, such findings indicate that the PS rat model has sufficient construct, face, and predictive validity to become an interesting "pathological" model for research on therapeutic approaches of depression-like disturbances.

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Environmental enrichment during adolescence reverses the effects of prenatal stress on anxiety-related behaviours and stress reactivity in rats

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Abstract

Prenatal stress (PS) can exert profound and long-lasting perturbations of an organism's adaptive capacities that can turn to an increased predisposition to behavioural disorders. Indeed, in PS rats there is evidence of increased anxiety and hypothalamus pituitary adrenal (HPA) axis disturbances. This study addressed the question of reversibility of such disturbances in PS adolescent male rats that were housed from weaning either in standard or in enriched physical conditions. As expected, PS rats showed reduced play behaviour, increased anxiety in the elevated plus maze and prolonged corticosterone secretion in response to restraint stress. Environmental enrichment increased play behaviour while decreasing environmental exploration in the social test, and it reduced risk assessment behaviour in the elevated plus maze although this was not associated with a concomitant increase of exploration of the open arms. PS enriched rats showed a reduced peak and a return to baseline levels similar to controls, thus indicating an improved regulation of the HPA axis. Interestingly, environmental enrichment had no effect on corticosterone secretion in the control group. As a whole, these results indicate that rats exposed prenatally to stress can benefit from the modulatory effects of an enriched environment during adolescence. Moreover they confirm that PS could represent a suitable animal model for the design and testing of new therapeutic strategies in behavioural disorders as a function of early insults.

Keywords: Prenatal stress, Enrichment, Adolescence, Corticosterone, Anxiety, Play behaviour

Introduction

There is general evidence that exposure to stress early in life can predispose individuals to the development of affective and anxiety disorders that persist through adulthood (Meijer 1985; Levitt et al., 1997; Gitau et al., 2001; Heim & Nemeroff 2001; for a review see Weinstock 1997). Fetal exposure to glucocorticoids may produce permanent hypertension, altered behaviour and neuroendocrine responses throughout the lifespan Seckl, 2001) These findings have suggested that such conditions may be in part prenatally programmed (Barker, 1995).

In rats, stressed dams during pregnancy can bear offspring with reduced expression of juvenile play (Ward & Stehm, 1991) and sexual performance in adult males (Ward, 1983), enhanced anxiety and emotional reactivity (Joffe, 1978; Vallee et al., 1997; 1999), in association with an impairment of the feedback inhibition of the hypothalamus-pituitary-adrenal (HPA) axis (Fride et al., 1986; Maccari et al., 1995; Vallee et al., 1997).

Although prenatal and postnatal events can have different consequences (Meaney et al., 1987; Vallee et al., 1999), they may also exert their influence on the same behavioural response. In this view, postnatal manipulations such as handling in the first three weeks of life (Wakshlak & Weinstock, 1990), or early postnatal adoption (Maccari et al., 1995) have been reported to reverse the effects of prenatal stress on emotional reactivity and HPA axis.

Several works have investigated the effects of environmental enrichment on brain/behavioural parameters in rat offspring exposed to different prenatal insults, such as alcohol (Hannigan et al., 1993), undernutrition (Carughi et al., 1990) or

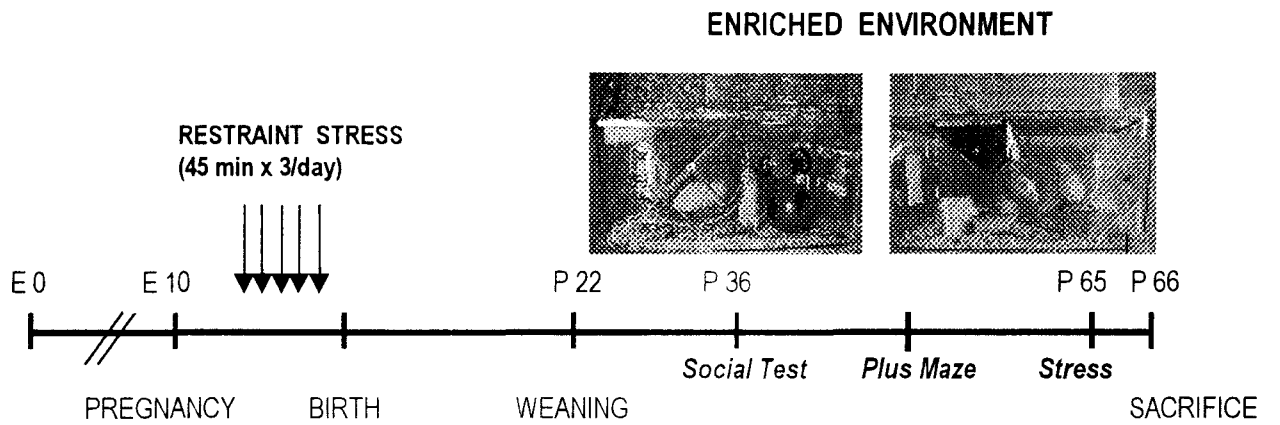

Fig. 1. Time line for experimental protocol showing age of animals (days) during each test. Enrichment condition consisted of differently shaped plastic containers, coloured platforms, suspended objects and a wheel. The objects were provided in three different sets that were changed twice a week. When the wheel was present the rats were provided with either a platform or a container suitable for hiding, the bottle of water was suspended above the ceiling and food pellets provided on the floor.

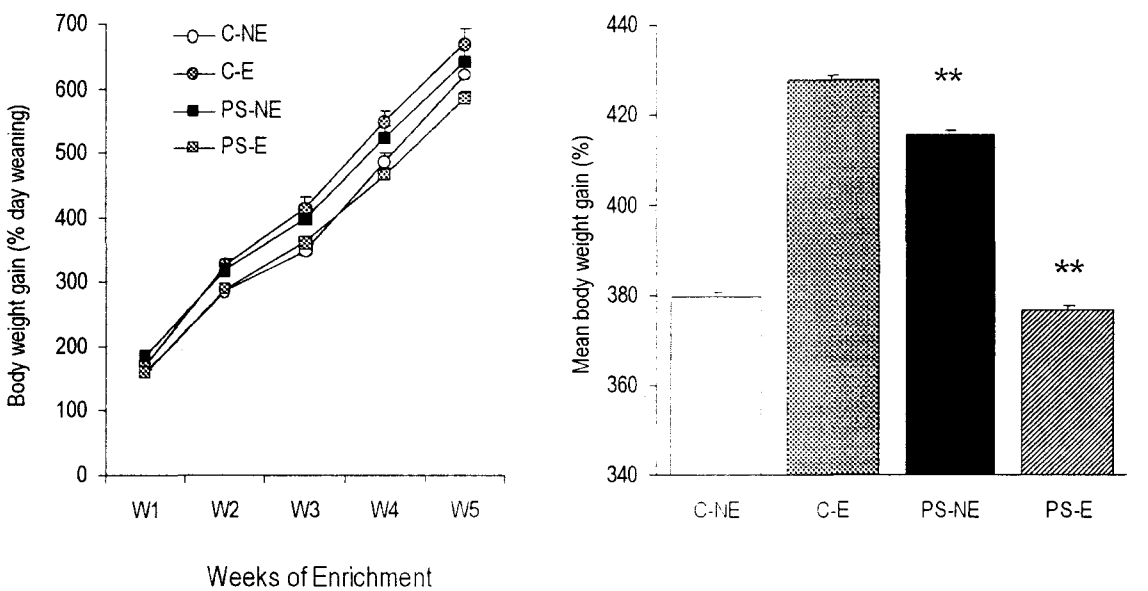

Fig. 2. Body weight gain. *Mean* (SEM) body weight gain expressed as percentage of day of weaning shown by periadolescent C and PS rats reared in standard (NE) or in enriched (E) conditions (n=11-14 for each group). **P < 0.01 vs Controls; ## P < 0.01 vs PS–NE.

neonatal anoxia (Iuvone, Geloso, et al., 1996). All these studies have reported beneficial effects of environmental enrichment with recovery of memory and learning capabilities.

One of the most robust effects of environmental enrichment on the behaviour of the rat appears indeed in the areas of learning and memory. However, whereas animals from enriched conditions clearly have better learning abilities than animals from standard conditions, the effects of environmental enrichment on emotionality are less documented and often inconsistent. For example, enriched rats were more active in an open field in some studies (Huck & Price, 1975) but not in others (Van Waas & Soffie, 1996; Pham et al., 1999). Enrichment was found to have inconsistent effects on several measures of emotionality in the Roman rat lines (Fernandez-Teruel et al., 1997), whereas it reduced the rat behavioural response to a predator (Klein et al., 1994). Furthermore, although environmental enrichment, like neonatal handling, has been associated with reduced glucocorticoid receptor expression in the hippocampus (Mohammed et al., 1993) there is less evidence for attenuated stress response in enriched reared rats (van Praag et al., 2000; Schrijver et al., 2002).

In the present study, we addressed the question of reversibility of PS-induced disturbances in male rats that where transferred at the time of weaning into conditions of either physical environmental enrichment or standard social housing. Animals were assessed during adolescence, an ontogenetic phase characterised by elevated basal levels of behavioural activation and a high expression of playful behavioural repertoire (Cirulli et al., 1996; Terranova et al., 1993; for a review see Laviola et al., 1999). Effects of enrichment were tested on social behaviour, anxiety in the elevated plus maze, and corticosterone secretion in response to restraint stress. Our results showed that environmental enrichment reversed the HPA axis reactivity to stress in PS rats and improved their behavioural repertoire.

Materials and methods

Animals

Sprague-Dawley female rats (250g) without prior breeding experience, were purchased from a commercial breeder (Charles River, Italy). Animals were housed in an air-conditioned room (temperature 21±1°C, relative humidity 60±10%), with a regular light/dark cycle (lights-on at 8.00 p.m.). Water and food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, Italy) were available *ad libitum*. For a week after arrival animals were group-housed (4 per cage) to coordinate their oestrus cycle. Females were then placed with a sexually experienced male and daily inspected for vaginal smear until the discovery of spermatozoids (designated as day of gestation 0), after which they were housed individually in Plexiglas cages (30x20x15 cm). Pregnant females were then randomly assigned to prenatal stressed (PS) or control (C) groups.

Prenatal stress procedure

Stress procedure started on day 11 of pregnancy until delivery at 21 days as previously reported (see Maccari et al., , 1995): pregnant females were individually placed in plastic transparent cylinders (diam. =7 cm., lenght =19 cm) and exposed to bright light for 45 min. Animals were submitted daily to three stress sessions starting at 09:00, 12:00 and 17:00 h, whereas control pregnant females were left undisturbed in their home cages. Male and female offspring were weaned on day 22 after birth, and only male offspring from litters containing 10-14 pups with a comparable number of males and females were used in the present study.

Housing and environmental enrichment

At weaning, pairs of sibling from the PS and C groups were assigned to each of the two rearing conditions. The standard laboratory conditions (NE) were defined as two animals housed in transparent cages (32x20x15 cm). Animals in the enriched condition (E) were housed in larger and higher cages (40x25x30 cm). Enrichment was then defined in terms of physical environment and not social housing condition (Renner & Rosenzweig, 1986), and consisted of differently shaped plastic containers, coloured platforms, suspended objects and a wheel (diam. = 15 cm). The objects were provided in three different sets, changed twice a week, in a way that animals had at the same time a protected and an open environment. When the wheel was present the rats were provided with either a platform or a container suitable for hiding, the bottle of water was suspended above the ceiling and food pellets provided on the floor.

For both E and NE conditions, the sawdust of the cage was changed once a week in association with measurement of body weight. In order to prevent litter effects, within each original litter no more than two male siblings were assigned to each condition (Chapman & Stern, 1979). Animals were put in the E or NE cages at the age of 23 days and maintained in their housing condition throughout all the experimental assessment.

Behavioural measures

Behavioural tests were carried out after a week of housing in an E or a NE condition, with no less than a three days intercourse between each test. The timeline (Fig. 1) illustrates the temporal sequence of events.

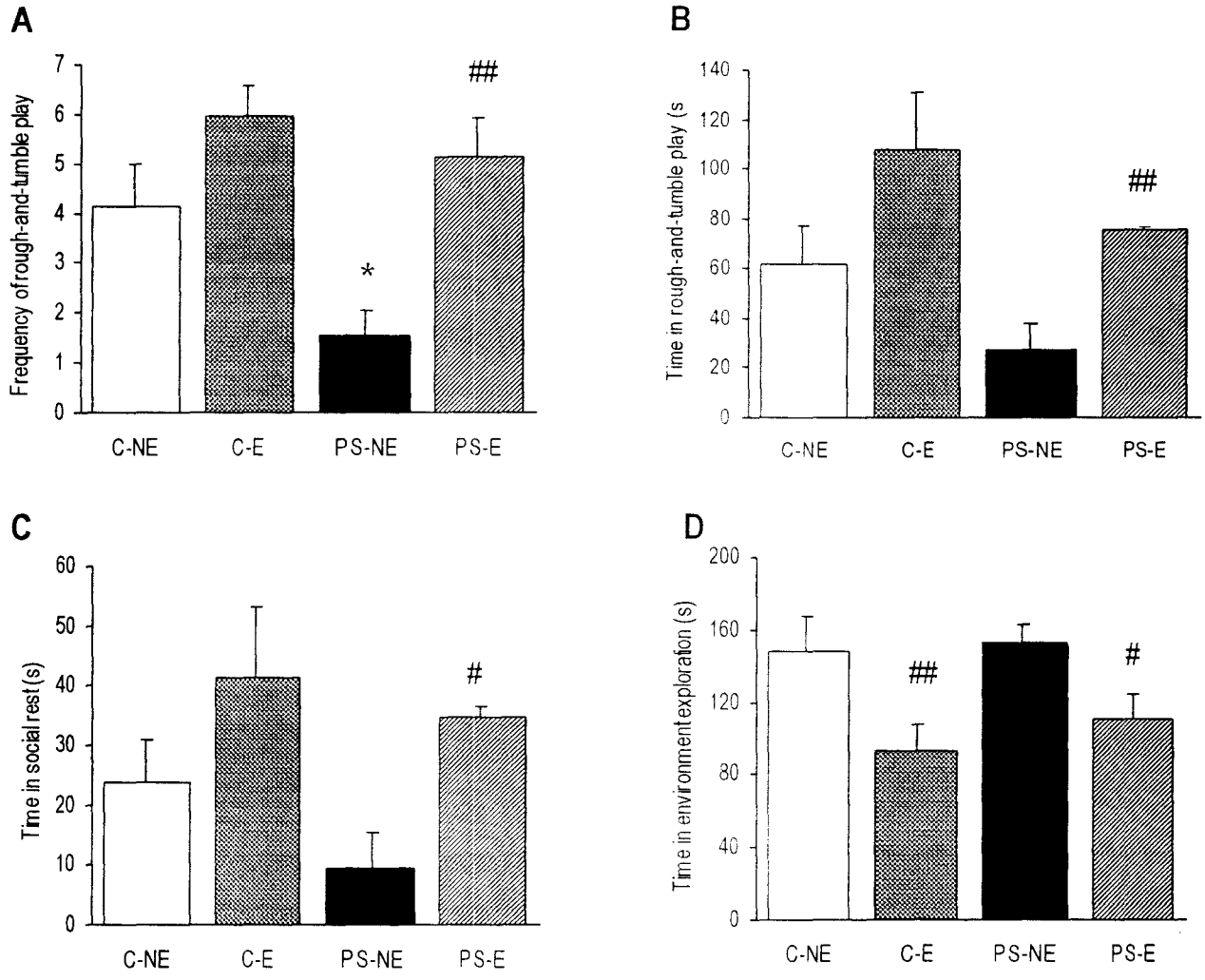

Fig. 3 Social interaction test. Mean (S.E.M.) frequency (3A) and duration (3B) of rough and tumble play, duration of social rest (3C) and of environmental exploration (3D), shown by periadolescent C and PS rats at 36 days of age reared in standard (NE) or in enriched (E) conditions (n=7 pairs for each group). *P < 0.05 vs C-NE ; #P < 0.05 and ## P < 0.01 NE vs E condition.

Social interaction test

In order to increase social behaviour rats were individually housed for 24 hours (Cirulli, et al., 1996; Terranova et al., 1999). Then, each pair of rats from the same cage and housing condition was placed in the test arena (40x25x30 cm) for a single 20-min session. All the session was video recorded using a professional Sony videocassette recorder VO-58OOPS apparatus. The whole session was automatically subdivided into five minutes intervals. The first and fourth 5 min of each session were manually scored (Observer 20 Noldus, Wageningen, The Netherlands) according to an "all-occurrence" sampling method (Martin & Bateson, 1986) by an observer blind to the assignment of animals to the different groups. Separate scores were obtained for each individual in a pair, but since the two values cannot be considered statistically independent, pair means were used for further analysis. The following social and non-social behavioural categories were recorded.

Social behaviours: *Investigative and affiliative elements*. 1) Social investigation (anogenital and body sniffing); 2) Allogrooming; 3) Close Following: the subject follow the partner around the cage; 4) Social rest: the subject is lying flat or standing still while maintaining physical contact with the partner, which may be in turn either inactive or involved in activities which do not require movements around the cage (i.e social sniffing or maintenance activities). This behaviour can elicit allogrooming by the partner. *Rough – and - tumble play*: pouncing (the subject lunges toward the side or back of the partner with the fore paws extended) wrestling and pinning (one of the animal lying with its dorsal surface on the floor with the other animal standing over it) with the partner. *Non-social behaviours*. 1) Explore: Sniffing the air, rearing and exploring the cage 2) Self-Grooming.
A total of six pairs of rats from each group and housing condition was used (total number of pairs = 24).

Elevated Plus Maze

The elevated plus maze was made of transparent Plexiglas, according to the specifications of (Cole & Rodgers, 1994). The apparatus consisted of two open arms (50x 10 cm), alternating at right angles, with two arms enclosed by 40 cm high walls. The four arms delimited a central area of 10 cm2. The whole apparatus was placed 1 m above the floor. The test was conducted under dim light and lasted 6 min, and began with the placement of the rat in the centre of the maze with the head facing an enclosed arm. The number of entries and the time spent in open and enclosed arms were recorded, and a four–paw criterion was used for arm entries. More ethological measures, like stretched attend postures (SAP, head dipping, grooming, rearing, and immobility in the closed arms were also scored (Rodgers & Johnson, 1995).

Radioimmunoassay for Corticosterone

At the end of the housing period, animals were assessed for the HPA axis response to a restrain stress. Briefly, blood samples were collected via the tail vein three times between 9:30 and 11:30 h. Rats were moved to an adjacent room and individually placed in a restraint transparent tube. Restraint was carried out in plastic cylinders identical to those used for the prenatal stress procedure. Blood was collected quickly (less than 1 min) to determine basal corticosterone levels(t0). A second sampling was performed 20 min after restraint stress was initiated (t20). Rats were then returned to their home cage with their usual partner, until the last blood sampling was performed 60 min after the initiation of the stress procedure(t60). Blood was collected in tubes filled with 100 µl of EDTA. Plasma corticosterone levels were obtained using a RIA Kit (ICN Biomedicals) with a highly specific corticosterone antibody and a detection threshold of 0.1 µg/100 ml. The intra- and inter-assay coefficients of variation were 5 and 11 % respectively.

Statistics

Data on social test and elevated plus maze were analysed using parametric analysis of variance (ANOVA) with two levels of group (prenatal stress vs controls) and two levels of condition (enriched vs non-enriched) as between subject factors (Winer, 1971; Chiarotti et al., 1987). Corticosterone levels were calculate by ANOVA with times of blood sampling considered as within subject factors and group and condition as between subject factors. The area under the curve (AUC) of corticosterone data was calculated by use of the trapezoidal rule. Planned contrast analysis were used for *post hoc* comparisons.

Results

Effects of prenatal stress on mothers and offspring at weaning

For mothers, ANOVA revealed a tendency for a reduced increment in body weight in stressed mothers when compared to control mothers ($F_{1,22} = 4.05$, $P = 0.056$ with 312 ± 4.5 grams stressed vs 328 ± 6.7 grams for controls). With respect to the offspring, prenatally stressed rats of both sexes were markedly heavier at weaning than controls ($F_{1,22} = 8.75$, $P < 0.01$) with about a 12% increment for males and 17% for females. No significant differences as a consequence of prenatal stress were found for the

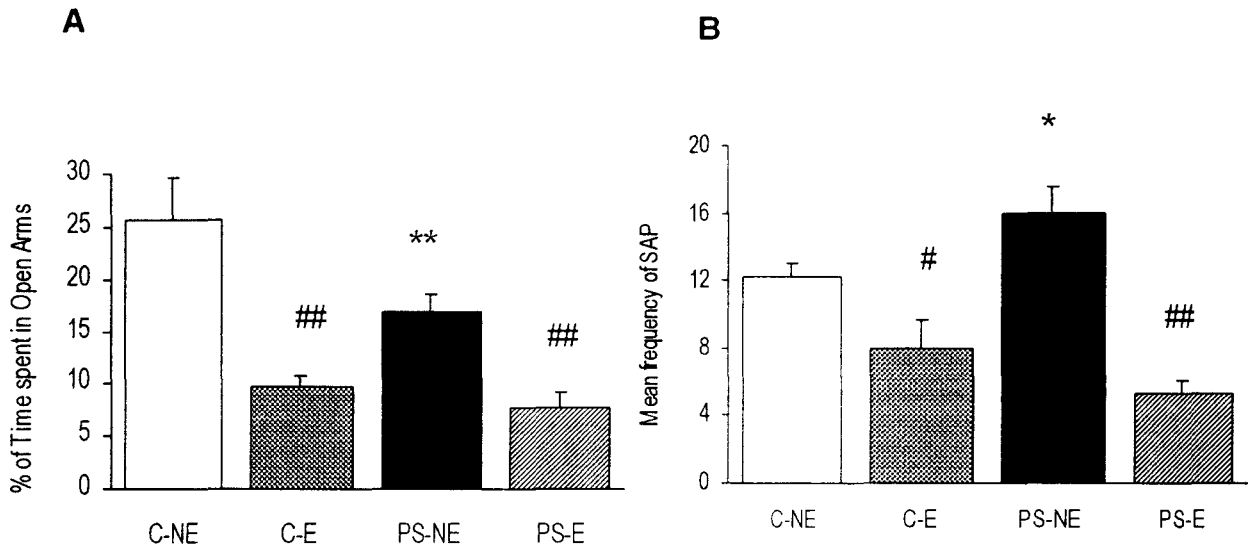

Fig. 4. Elevated Plus Maze test. 4A. Percentage of time spent in open arms (open/open + closed) measured in the elevated plus maze over 6 min test shown by periadolescent C and PS rats at 41 days of age reared in standard (NE) or in enriched (E) conditions. 4B. Frequency of stretched attends postures (SAP) shown by the same animals (n=8-10 for each group). *P < 0.05 and ** P < 0.01 PS-NE vs C-NE ; #P < 0.05 and ## P < 0.01 NE vs E condition.

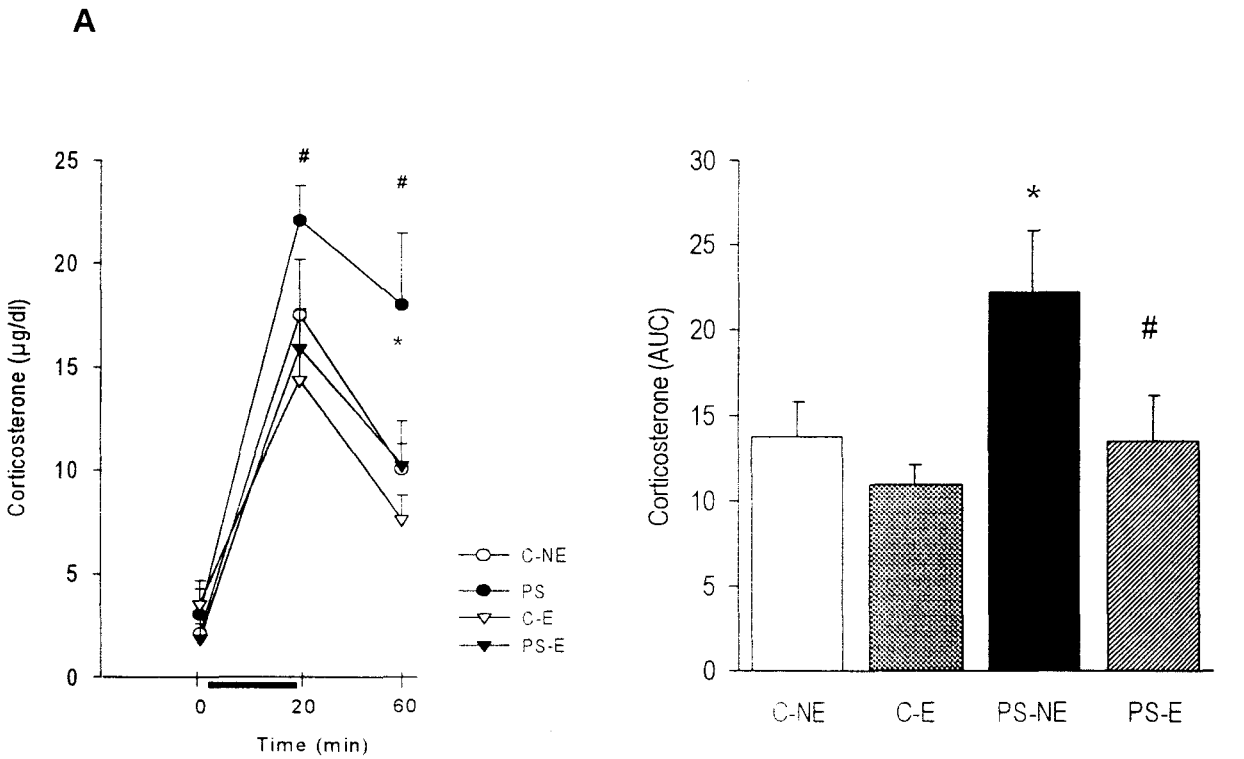

Fig. 5 Stress-induced corticosterone secretion 5A. Mean (S.E.M.) plasma corticosterone secretion (µg/dl) in basal condition (time, 0 min) and in response to a 20 min period of restraint stress (time 20, and 60 return to baseline) shown by periadolescent C and PS rats at 60 days of age reared in standard (NE) or in enriched (E) conditions. 5B. Mean (SEM) area under the curve (AUC) analysis for the corticosterone data displayed in 5A calculated by using the trapezoidal rule (n=11-14 for each group). *P < 0.05 PS-NE vs C-NE ; # P < 0.05 vs PS-E group.

length of gestation (22 days), number of pups (about 13 per litter), and sex ratio (M/F=1).

Body weight gain

Results are shown on Fig. 2. Offspring body weight gain, expressed as percentage of body weigh at weaning, was measured weekly. A group by condition interaction ($F_{1,42}$ = 14.80, P < 0.01) revealed that within the standard condition PS animals were constantly heavier than controls ($P < 0.01$) throughout the five weeks of housing. In contrast, this profile was opposite in the enriched condition. As a consequence PS rats raised in enriched environment showed a body weight gain similar to control non-enriched rats.

Social Interaction Test

Rough - and - tumble play (Fig. 3 A, 3B): a main effect of group was found for both frequency ($F_{1,21}$ = 5.74 $P < 0.05$) and duration ($F_{1,21}$ = 4.11 $P < 0.05$) indicating reduced amounts of play shown in general by PS animals. Interestingly, levels of play were markedly increased following enrichment procedure (condition, $F_{1,21}$ = 8.26 $P < 0.001$; and $F_{1,21}$ = 3.35 $P < 0.001$, for duration and frequency respectively). PS rats reared in the enriched condition showed levels of play similar to controls ($P < 0.05$ and $P < 0.01$ vs PS standard, for duration and frequency respectively).

Affiliative behaviour: With respect to carry over effects of prenatal stress important alterations were detectable only on in subjects reared in standard conditions. A separate analysis conducted on this set of data, evidenced a main effect of group for social investigation ($F_{1,22}$ = 4.42, $P < 0.05$) and a trend for allogrooming ($F_{1,22}$ = 3.77, $P = 0.06$) with PS rats showing in general higher levels than control). Environmental enrichment was responsible for an increment in the duration of social rest behaviour ($F_{1,22}$ = 6.59, $P < 0.01$; Fig. 3C) whereas it reduced the duration of social investigation ($F_{1,22}$ = 3.38, $P < 0.01$), and follow ($F_{1,22}$ = 5.17, $P < 0.05$).

Non social behaviours: Time spent exploring the cage ($F_{1,22}$ = 10.69, $P < 0.01$; Fig. 3D) was significantly reduced in enriched animals and a trend for a similar profile for levels of self-grooming was also noticed.

Elevated Plus Maze

Results are shown in Fig. 4. The percentage of time spent in the open arms was significantly higher for controls than for PS group (group, $F_{1,29}$= 6.15, $P < 0.01$; Fig. 4A).

Significant differences were also observed according to housing conditions in the elevated plus maze. Overall, E animals spent less time than NE ones in the open arms (condition, $F_{1,29}$ = 28.30, $P < 0.001$). A general score of locomotor activity, namely the number of closed arm entries was lower in prenatally stressed rats both in E and NE condition (group, $F_{1,29}$ = 6.97, $P < 0.01$ and condition, $F_{1,29}$ = 6.20, $P < 0.01$).

The frequency of stretched attending posture (SAP) was significantly reduced in enriched rats and this effect was much higher in the PS group (P < 0.001 vs standard) as revealed by the group by condition interaction ($F_{1,29}$ =8.63, $P < 0.01$; Fig. 4B).

Basal and stress-induced corticosterone secretion

Plasma corticosterone response as measured immediately before, during and up to 60 min after the restraint procedure, is shown in Fig. 5A. As a whole, main effects of condition and time were found ($F_{1,31}$ = 4.91, $P < 0.05$; $F_{2,62}$ =63.66, $P < 0.01$ respectively), with enriched animals showing reduced levels of corticosterone throughout the sampling period and that reached significance 20 min after the stress ($P < 0.05$ vs NE). A separate analysis was then performed for each time point of sampling. Analysis of basal level (t0) revealed no effect of prenatal stress (group, $F_{1,41}$ = 0.12 n.s) or housing condition ($F_{1,41}$ = 1.53, n.s), whereas at the peak level (t20) enrichment was responsible for a significant reduction of corticosterone secretion in prenatal stressed animals and not in controls ($F_{1,41}$ = 5.28, $P < 0.05$). As expected, at t60 a significant elevation in plasma corticosterone was observed in PS-NE rats compared to controls (group, $F_{1,31}$ = 5.47, $P < 0.05$). Enrichment procedure had no effect on controls whereas on PS it induced a more rapid return to baseline levels equal to control rats (condition, $F_{1,31}$ = 5.19, $P < 0.05$). When considering the area under the curve (AUC; Fig. 5B), the integrated levels of corticosterone were higher in PS-NE rats compared to controls (group, $F_{1,31}$ = 4.8, $P < 0.05$) and to the corresponding enriched group (condition, $F_{1,31}$ = 5.12, $P < 0.05$).

Discussion

This study was aimed to investigate the interplay between prenatal stress and a postnatal manipulation performed throughout the adolescent period such as enrichment of the environment. Previous studies on consequences of prenatal stress have been mostly carried on adult subjects. The present study provides literature a first more complete behavioural characterisation of some aspects of the emotionality of the adolescent PS rat that need to be considered

first separately.

Carry-over effects of prenatal stress on the behavioural and endocrine outcome in the adolescent rat

PS rats engaged more time in investigative behaviours than control animals whereas exhibited a reduced amount of rough-and-tumble play both in terms of duration and of frequency. These data confirm and extend the findings previously obtained by (Ward & Weisz, 1984). Interestingly, we obtained almost the same results although the procedure adopted was quite different, and implicated different considerations. In the above mentioned study, one focal animal was observed while interacting with unrelated litter mates in the home cage. Whereas in our study, a pair of sibling from the same housing condition was confronted in a novel environment. The results obtained with the first procedure reported PS-related reduction of basal levels of play behaviour in a group of unrelated individuals. The paradigm adopted in the present study assessed the social behavioural strategy adopted by adolescent rats when faced to a novel environment in a pair of siblings. In this experimental condition control animals exhibited the expected increment of social play behaviour as a coping strategy to novelty. On the other hand, PS animals spent much more time sniffing and investigating their partner.

In the elevated plus maze, PS animals showed elevated levels of anxiety as indicated by the reduced amount of time spent in the open arms. This profile was also associated with an increased frequency of risk assessment stretched-attend postures (SAP). These data confirm the profile previously reported for adult PS rats in the same experimental paradigm (Vallee et al., 1997; 1999) as well as in others tests of emotionality (Fride & Weinstock, 1988; Wakshlak & Weinstock, 1990).

From an evolutionary perspective, the general behavioural profile observed as a consequence of prenatal stress already at the adolescent stage may have important implications for the development of maladaptive behaviours and the successful survival of the individual in a social setting. Rats in the age range from around 28 to 42 days are often hyperactive and exhibit greater exploration in novel situation than subjects from other age groups (Spear et al., 1980); for this profile in mice see Adriani et al., 1998). These specific age-related increases in exploration and novelty seeking, together with the adolescent associated elevation in social affiliative interactions may help adolescent rodents to successfully negotiate the developmental transition from dependence to independence (for a review see Laviola et al., 1999; Spear, 2000).

In agreement with the report of Dugovic and coworkers (1999), peripubertal PS rats presented a higher peak of levels of stress-induced corticosterone secretion and retarded return to the baseline following the extinction stress (t60) when compared to controls. These results then confirm the profile of impaired feedback inhibition of HPA axis activity caused by maternal stress, and evidenced in the offspring throughout the different phases of ontogenesis (Henry et al., 1994).

With respect to measure of body weight gain, our results indicated that PS rats were consistently heavier than controls reared in the standard condition. On this issue, conflicting results are available in the literature. Some authors have reported a reduction of body weight in PS compared to control rats (Cabrera et al., 1999; Pollard, 1984; Vallee et al., 1996), whereas others have reported an opposite profile (Pfister & Muir, 1992). A simple explanation may implicate an alteration in feeding behaviour although the mechanisms involved remains to be elucidated. A previous work by Vallee and colleagues (1996) suggested that prenatal stress may influence metabolic set points through a lower food intake associated with an increased amount of blood glucose levels. In this regard, discordances on the effects of prenatal stress on body weight gain should take into account also differences in the developmental stage.

Interplay between environmental enrichment during adolescence and prenatal stress

The experience of a physical enriched environment resulted associated with a significant increment of body weight gain in non-stressed animals. A possible explanation for this observation could be an improved regulation of body temperature in enriched rats. The occurrence of groups resting inside the toy objects was frequent, which may reduce heat loss (see Van de Weerd et al., 1997). Such effect was much less evident in PS animals housed in enriched condition which in fact gained weight more slowly. The profile of these animals resembled that of control animals housed in standard conditions. As a whole, this result indicates that environmental enrichment exerted its influence on the regulation of body weight differentially as a function of prenatal background.

Following environmental enrichment PS rats showed a significant increase in levels of rough and tumble play together with a decrease in environmental exploration. As a consequence their amount of play was similar to control rats reared in standard condition. Expression of social play early in life is necessary for an adequate development of appropriate response in social situations later in life. It is

interesting to note that while several studies have investigated effects of enrichment on memory function and anxiety, up to date to our knowledge very few studies (see Renner & Rosenzweigh, 1986) have addressed the issue of its influence on social behaviour. Play behaviour is associated with a high risk of exposure to predators, and engaging in vigorous form of social interaction might be accompanied by lower levels of attention of the environment. If the environmental conditions are such that reduced attention is not possible, animals will not engage in play. Therefore, the higher amount of play behaviour observed in enriched animals could implicate a reduced profile of anxiety.

In this regard, the reduced exploration of the open arms in the elevated plus maze observed in enriched rats would appear in contrast with a reduced profile of anxiety if the profile of risk assessment behaviour wouldn't have been scored at the same time. Enriched rats explored significantly less the open arms, but their exploration of the apparatus was characterised by a significant reduction of SAP. This behaviour was described as an approach-avoidance conflict and was expressed as part of a risk assessment factor in the elevated plus maze (Rodgers & Johnson, 1995). PS animals were characterised by a significantly higher frequency of SAP which was markedly reduced by enrichment. Cruz and coworkers (1994), by conducting a factor analysis on plus-maze behaviour, indicated that an elevated frequency of SAP was shown only in association with the experience of high levels of anxiety. Although the exploration of the open arms was also reduced, fewer SAP could then lead to hypothesise that the motivation to explore was increased (for similar results in mice see Roy et al., 2001). Further studies will better investigate this point to support such hypothesis by also allowing animals explore for a longer time (i.e. more than 6 min) the elevated plus-maze.

The effects of enrichment on reactivity to stress and thus on stress-induced corticosterone secretion were on the contrary very clear. PS rats reared in an enriched environment showed a reduced peak and a return to baseline levels similar to controls, thus indicating an improved regulation of the HPA axis. The present study provided also evidence that in control subjects levels of secreted corticosterone in response to acute restraint challenge were unaffected by environmental background. In fact, peak and recovery levels were very consistent for both standard and enriched-reared groups. In contrast, important effects were observed in PS animals. It seems possible that at least for this physiological parameter a postnatal manipulation with a positive qualitative intervention such as enrichment of the physical environment was able to exert its influence only on more responsive perhaps because more vulnerable individuals such as those from the PS offspring. Indeed it is important to underlie that the negative results reported in literature on the interplay between corticosterone and enrichment concern studies conducted mostly on fully healthy animals, not previously manipulated (van Praag et al., 2000; Schrijver et al., 2002). In this line, a very recent work of Francis and colleagues (2002) has reported reversal by enrichment of behavioural and HPA axis reactivity to stress in maternally deprived rats but not in handled rats. It is then interesting to note that in humans, programs of child intervention which serve to offset the risk associated with family stress for intellectual and emotional development have reported that such effects are most apparent in individuals whose development was compromised as a function of early adversity (Ramey & Ramey, 1998).

As a whole, the positive effects of enriched environment on PS rats parallel and extend those previously reported with other postnatal manipulations on stressed animals such as the effects of early adoption on HPA axis (Maccari et al., 1995), and neonatal handling on anxiety-like behaviour (Wakshlak & Weinstock, 1990). However, a major difference between our postnatal manipulation and the postnatal intervention previously reported on PS rats is the fact that they were carried in the early postnatal period. In this regard, we chose not to expose the dam with their pups to enriched environment because preweaning enrichment can interfere with early postnatal development thereby affecting the relationship between dam and pups. In fact, it has been shown that enrichment conditions for the mothers have effects on their offspring (Dell & Rose, 1987). Also, in postweaning enrichment animals actively explore their new environment, thus actively pursuing the sensory stimulation, whereas in preweaning enrichment pup animals are more passive in experiencing the stimuli (Kohl et al., 2002). For this issue, effects of enrichment on neurogenesis could also be taken into account. Indeed it has been shown that postweaning enrichment, but not preweaning one (Kohl et al., 2002), enhances hippocampal neurogenesis in the adult animals (Kemperman et al., 1997; Van Praag et al., 1999), and prenatal stress has been shown to induce lifespan reduction of neurogenesis in the hippocampus (Lemaire et al., 2000).

In the present study, rats exposed prenatally to stress benefited from the effects of an enriched environment during the still plastic period of adolescence. It is important to note that the positive effects reported in this work were obtained with an enrichment procedure which adopted cages of

laboratory size, representing then a housing practice that can be easily standardised. Indeed, it may be questioned whether standard housing conditions provide an ideal environmental background for the study of complex brain functions. In fact, in the laboratory, the safety and stability of the rearing environment does not reliably predict the future challenges involved in life as an experimental animal (Wurbel, 2001). A better understanding of environmental factors that are involved in control of behaviour, together with an intuitive enrichment of that environment, might be of great benefit to both research and animals.

Abbreviations

C, control (group); PS, prenatal stress (group); HPA, Hypothalamus-pituitary-adrenal-axis; NE, non-enriched; E, enriched.

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DISCUSSION GENERALE

DISCUSSION GENERALE

Cette thèse avait pour but d'évaluer la validité d'un modèle animal dans l'étude de la vulnérabilité aux drogues et aux troubles dépressifs. Deux modèles différents d'expérience précoce ont été adoptés. Le premier est un phénomène naturel : la position intra-utérine, alors que le second est un phénomène induit : l'exposition à un stress prénatal. Nous avons montré que les deux modèles avaient des effets à long terme sur la vulnérabilité aux drogues, le comportement et plus généralement, les stratégies d'adaptation (« *coping* ») face aux conditions environnementales.

Nous avons également examiné l'efficacité de deux stratégies d'intervention chez la descendance pour reverser les altérations induites par le stress maternel : une stratégie pharmacologique et une stratégie environnementale. Les deux paradigmes ont prouvé leur efficacité dans le réversion les altérations comportementales, endocriniennes et neurochimiques induites par le SP.

A. L'ENVIRONNEMENT PRECOCE ET LA VULNERABILITE AUX DROGUES

La position intra-utérine est un modèle naturel de changements hormonaux qui peuvent interférer avec la sensibilité différentielle aux psychostimulants.

La PIU joue un rôle essentiel dans la variabilité qui n'a pas d'origine génétique. Chez les mammifères, la PIU influence fortement la masculinisation ou la féminisation physiologique, morphologique et comportementale. En outre, la PIU exercerait des effets importants au niveau de la population d'une espèce (Cowell et al., 1998). En réponse à la modulation de la PIU, les individus présentent des capacités reproductrices altérées et une propension à montrer de l'agressivité ou de la dispersion. Ces facteurs fournissent une population avec une variabilité phénotypique qui lui permet de s'adapter rapidement aux changements même si les individus de la population sont génétiquement homogènes.

L'exposition à la testostérone lors de la vie précoce augmente le profil de recherche de nouveauté, chez le mâle, et induit une plus grande sensibilité à l'administration d'un agoniste de la morphine, dans les deux sexes. Ces résultats confirment les résultats précédents montrant une augmentation de la sensibilité à la testostérone chez les sujets 2M. Comme décrit précédemment, les souris mâles et femelles de phénotype 2M requièrent un traitement plus court à la testostérone pour induire l'agressivité (vom Saal, 1984), et les rats femelles 2M deviennent stériles plus rapidement et anovulatoires plus tôt que les femelles 0M après l'injection de testostérone (Tobet et al., 1982). Nos résultas indiquent que l'exposition prénatale à différents taux d'hormones sexuelles est capable de moduler les processus

d'organisation précoce de systèmes cérébraux qui sous-tiendront l'expression du comportement de recherche de nouveauté à l'adolescence. Un développement dans des conditions 2M peut avoir des conséquences sur la façon qu'a la souris, autour de la puberté, de s'adapter à son environnement.

Il est important de souligner que, bien que l'exposition à la testostérone semble augmenter la sensibilité à l'action des psychostimulants, le phénotype 0M devrait être considéré comme étant un profil plus vulnérable, étant donné qu'il est le phénotype le plus sensible à une situation stressante. En fait, l'exposition à un stress lors de la vie prénatale affecte le développement des sujets 0M et non celui des sujets 2M, induisant un déplacement de profil de toutes les PIU vers le profil 2M (vom Saal, 1984; vom Saal et al., 1990). Cela élimine la variabilité dans la population et avantage les phénotypes plus sensibles aux effets de la testostérone, augmentant ainsi la proportion des individus susceptibles de développer une sensibilité accrue aux psychostimulants.

Il y a des preuves directes que le SP, bien que perturbant la différentiation sexuelle, interfère aussi avec l'expression du système opioïde chez la descendance. Par exemple, le nombre de récepteurs aux opioïdes mu dans le striatum sont réduits chez les rates femelles (Insel et al., 1990) et les effets analgésiques de la morphine sont diminués chez les mâles et augmentés chez les femelles (Kinsley et al., 1988). Le stress maternel réduit la distance anogénitale et interfère avec l'activité sexuelle chez les mâles (Ward, 1972) et ces changements sont également observables après injection d'opiacées (Ward, 1983; Tempel et al., 1988). Des effets du SP en parallèle de ceux décrits dans notre étude , menant à l'hypothèse que, en terme de PIU, un déplacement vers le phénotype 2M se produit. En effet, les sujets 2M présentent une activité sexuelle réduite et ont montré une réduction des récepteurs μ dans le striatum chez les mâles et une augmentation de la sensibilité aux effets analgésiques de la morphine, chez les deux sexes.

De façon intéressante, les effets de la PIU ont aussi le potentiel d'influencer les études toxicologiques. La perturbation endocrinienne est un domaine qui reçoit une grande attention, comme il s'agit d'un domaine de recherche qui cible les faibles doses de produits chimiques qui peuvent entraîner des effets toxiques chroniques (vom Saal et al., 1995). Le plastique et les pesticides sont des exemples de produits qui contiennent substances entraînant des perturbations endocriniennes au niveau des œstrogènes qui peuvent interférer avec le développement des mammifères en mimant les actions des œstradiols (Colborn et al., 1993). Par exemple, l'exposition à de fortes doses de substances perturbatrices du système endocrinien peuvent induire des anormalités génitales chez les garçons (Paulozzi et al. 1997) aussi bien qu'une maturation sexuelle plus précoce chez les filles (Herman-Giddens et al., 1997). Il a été montré que des changements subtiles des hormones sexuelles induites par la PIU peut directement influencer la sensibilité à certains

perturbateurs endocriniens. Howdeshell et ses collaborateurs (1999) ont mené une étude traitant les souris femelles 0M et 2M prénatalement avec de faibles doses de bisphenol-A oestrogénique environnemental. C'est un composé qui est utilisé pour la production de polycarbonates dans les biberons et dont les taux augmentent à chaque (Brotons, 1995). Les femelles 0M semblent être plus sensibles au busphénol-A que les femelles 2M, car quet elles sont exposées à ce produit chimique prénatalement, les femelles 0M montrent un plus court intervalle entre l'ouverture du vagin et le premier œstrus, ce qui indique une accélération marquée de la puberté. Ainsi, de très petites augmentations dans les nivaux d'œstradiol endogène peuvent augmenter de façon substantielle la sensibilité des fœtus à des perturbateurs endocriniens consommés par les femmes enceintes, donc des fœtus peuvent présenter des risques particulièrement importants pour des pathologies variées.

La variabilité est typiquement contrôlée en standardisant le contexte génétique des sujets et l'environnement dans lequel ils sont maintenus après la naissance. La PIU est une autre source de variabilité rarement reconnue. Cette variabilité n'est ni génétique, ni environnementale, mais plutôt hormonale. Nos données provenant d'un modèle animal, indiquent que les individus exposés à des variations subtiles d'hormones sexuelles endogènes au cours de la vie prénatale résultant de la PIU, peuvent montrer une sensibilité particulière au cours de l'adolescence incluant une augmentation de la vulnérabilité aux drogues psychoactives.

L'autre étude conduite au cours de l'adolescence a envisagé un modèle induit de variations hormonales, tel que le modèle de stress prénatal. Les rats stressés prénatalement ont montré une augmentation de la réponse au MDMA au cours de l'adolescence au niveau comportemental, mais aussi métabolique. Les différences observées entre les rats SP et les rats contrôles traités avec la même quantité de drogue suggèrent aussi qu'il y a a des différences individuelles dans les taux sanguins de drogue qui jouent un rôle majeur dans les effets induits par les drogues à la place de la dose de drogue elle-même. Ce résultat avec ceux d'autres rapports indiquant un taux de glucose basal augmenté dans le sang chez les animaux adultes SP (Vallee et al., 1996) ainsi que la preuve récente d'un profil d'intolérance au glucose et en même temps de résistance à l'insuline chez les rats âgés (Lesage et al., 2002) souligne les niveaux d'impact multiples induits par une exposition à un stress précoce. Un autre point est que notre étude a été conduite chez les femelles alors toutes les autres études conduites précédemment sur les rats SP étaient conduites chez des mâles (Deminiere et al., 1992; Koehl et al., 2000). Bien que les différences sexuelles dans la pharmacocinétique ont été conduites chez l'homme (Fletcher et al., 1994), cet aspect n'a pas reçu beaucoup d'attention dans les études pharmacologiques humaines et expérimentales chez l'animal pour expliquer la variabilité dans la vulnérabilité aux drogues (Lynch et al.,

2002). Etant donné que le SP affecte la différentiation sexuelle (Ward, 1983), le résultat de possibles altérations dans les différences sexuelles dans la pharmacocinétiques pourrait prise en compte dans de futures recherches.

Lorsque nous transférons les résultas du traitement à la drogue des situations cliniques aux animaux de laboratoire, peu d'attention a été accordée aux différences spécifiques des espèces potentielles dans le métabolisme de la drogue appliquée. Dans ce contexte, en regard des différences observées entre les groupes SP et contrôles, notre étude a révélé que les concentrations sanguines obtenues chez les rats étaient contenues dans l'éventail des consommateurs humains de MDMA (Hemlin et al., 1996), indiquant ainsi que l'utilisation de rongeurs adolescents peut vraiment constituer un modèle approprié et utile pour examiner les mécanismes sous-tendant l'abus de drogues.

De façon intéressante, les différences liées au sexe observées chez les adolescents et les adultes pourraient être liées en partie aux différences associées à la pharmacocinétique (Spear, 2000). Les changements dans la composition du corps et la fonction des organes avec la croissance soudaine à l'adolescence et la hausse des stéroïdes gonadiques pourrait altérer le métabolisme et le taux d'excrétion des drogues (Heink, 1987).

Ainsi, les glucocorticoïdes programmateurs affecte la réponse à la drogue plus tard dans la vie. Dans ce contexte, il a été prouvé que la corticostérone elle-même est renforçatrice et que les rongeurs se l'auto-administrent, que ce soit par voie intraveineuse ou par voie orale (Piazza et al., 1996). La corticostérone est vue comme un psychostimulant endogène dépendant de l'état de l'animal qui a en commun beaucoup des effets neurochimiques et physiologiques avec des drogues psychostimulantes telles que l'amphétamine et la cocaïne(Piazza et Le Moal, 1996). Dans ce contexte , il serait très significatif que plusieurs études ont reporté que les rats adolescents montrent des augmentations de corticostérone plasmatique induites par la drogue, plus faibles que les adultes après un test à l'éthanol (Silveri et Spear, 1998) ou à l'amphétamine (Adriani et al., 1998; Laviola et al., 1999). Aussi loin que les élévations de la corticostérone contribuent à la valeur de récompense des drogues, de hauts niveaux d'exposition aux drogues chez les adolescents pourraient être nécessaire pour atteindre la valeur renforçatrice que ces drogues ont chez les adultes. Cette notion implique que, cependant, que les valeurs de récompense pourrait progressivement augmenter avec les niveaux croissants de corticostérone , ce qui pourrait ne pas être le cas dans les études conduites chez l'animal. dans ce cas, les augmentations inférieures d'un seuil de corticostérone nécessaire pour supporter l'auto-administration de drogue pourrait ne pas mener nécessairement à une augmentation de la consommation de drogue (Spear, 2000).

Ces considérations soulignent la nécessité d'examiner les différences éventuelles liées à l'âge dans la réponse aux drogues chez les animaux SP au niveau neuroendocrinien et

comportemental.

B. EVALUATION DU STRESS PRENATAL CHEZ LE RAT COMME MODELE DE LA DEPRESSION

Nous avons observé que le comportement d'immobilité dans le test de la nage forcée était corrélé avec les taux de corticostérone et que le traitement aux antidépresseurs diminuait l'immobilité chez les rats SP.

Dans ce contexte, nos résultats sur la corticostérone et l'immobilité dans le test de la ange forcée confirment ceux d'études précédentes indiquant que ce comportement est sensible aux stéroïdes surrénaliens. Une étude menée par Garcia-Marquez et Armario (1987) a démontré que chez deux modèles de stress (choc électrique chronique ou stress aigu), l'exposition chronique, mais pas aiguë aux stresseurs induit une augmentation de l'immobilité. Il y a plusieurs évidences d'une implication directe des taux élevés de corticostérone dans cette réponse comportementale qui est cohérente avec nos résultats et ceux de Alonso et ses collaborateurs chez les femelles et avec un autre modèle de SP (Alonso et al., 1999; 2000).

De kloet et ses collaborateurs (1988) ont montré que le RU38486 (antagoniste GR) administré au niveau de l'hippocampe dorsal avant le test initial bloque la rétention de l'immobilité. De plus, la surrénalectomie diminue l'immobilité alors que l'administration de corticostérone l'augmente (Mitchell et Meaney, 1991) et bloquant la synthèse de corticostérone avec la metapyrone 3 heures avant le test de nage forcée , diminue la réponse d'immobilité (Baez et Volosin, 1994). Une souris *knock out* partielle pour le GR montre aussi une diminution de l'immobilité et un effet similaire apparaît avant l'injection d'un antisens des récepteurs aux glucocorticoïdes dans l'hippocampe avant le test (Korte et al., 1996). Collectivement, ces résultats suggèrent que des taux élevés de corticostérone sont nécessaires pour impliquer le mécanisme des GRs.

Nous avons observé une augmentation de l'immobilité après le SP que nous supposons être médiée par l'augmentation de corticostérone maternel. L'inhibition de la 11 βHSD 2 par la carbenoxolone augmente les niveaux de base de corticostérone chez l'adulte et diminue le comportement d'immobilité dans le test de la ange forcée chez la descendance (Welberg et al., 2000). Le même profil a été reporté lorsqu'on traite la mère avec de la dexaméthasone lors de la dernière semaine de gestation (Welberg et al., 2001). Ces résultats sont surprenants étant donné que le traitement prénatal décrit dans ces deux études comme le paradigme de SP résulte, chez le rat, en une altération comportementale, une augmentation de l'anxiété et une augmentation de l'activité de l'axe HHS.

Le test de la nage forcée est un paradigme comportemental qui est utilisé dans le *screening* de l'activité antidépressive chez le rongeur (Porsolt, 1978). Bien que ce paradigme possède un des plus hauts degré de validité prédictive en terme d'identification d'antidépresseur, un problème subsiste quant à l'interprétation de l'immobilité lors du test de la nage forcée comme reflétant une altération de l'adaptation et donc, un test approprié pour le comportement animal ressemblant à la dépression. Il a été proposé que l'immobilité pourrait également être vue comme une adaptation comportementale dont le but serait la conservation énergétique dans une situation de laquelle l'animal ne peut s'échapper (West, 1990). Dans ce cadre, et en dépit des taux élevés en période prénatale te faibles à l'âge adulte de corticostérone des niveaux diminués du comportement d'immobilité ont été interprétés comme une stratégie d'adaptation moins stressante à une situation aversive. Nous pensons que le comportement d'immobilité observé dans nos études chez le rat SP pourrait être du à un augmentation de la réponse de la corticostérone après une exposition au stress tel que la nage forcée.

Dans nos études, nous nous sommes intéressés à l'utilisation de ce test principalement pour son efficacité dans le *screening* d'antidépresseurs. Ainsi, le test de la nage forcée ne détecte pas seulement les antidépresseurs avec un comportement commun (immobilité) mais, des profils de comportement actif dans ce test , tel que le comportement d'agrippement aux parois et la nage révèlent de multiples composants des réponses qui sont sensibles aux différentes classes d'antidépresseurs (Lucki, 1997). Par exemple, les ISRS augmentent le comportement de nage, alors que les drogues agissant sur la sérotonine ou la noradrénaline, augment le comportement d'agrippement aux parois. La réponse des rats SP au traitement chronique avec la tianeptine ou l'imipramine, induisant respectivement une augmentation de la nage ou de l'agrippement aux parois, pourrait souligner l'implication du système sérotoninergique dans ce comportement (Moser et Sanger, 1999) et nous avons montré une dysfonction du système sérotoninergique chez les rats SP (article n°5).

Cependant, d'autres facteurs que les glucocorticoïdes devraient être pris en compte dans cette réponse comportementale. Une étude conduite par Molina et ses collaborateurs (1994) a exploré la participation du système opioïde endogène dans l'immobilité. Cette étude reporte une augmentation de l'immobilité chez les rats soumis à un stress chronique aussi bien que chez les rats traités de façon chronique à la morphine. Dans les deux groupes, cette immobilité potentialisée était atténuée par un pré-traitement à la naloxone. Dans ce contexte, bien que cette hypothèse n'ait pas encore été examinée, les altérations du système opioïde reportées précédemment chez les rats SP (Poltyrev et Weinstock, 1997) pourraient être un facteur additionnel expliquant leur comportement dans notre travail, en accord avec les autres anormalités comportementales dépendantes des opiacées et déjà décrites chez ces animaux (Keshet et Weinstock, 1995; Kinsley et al., 1996; Weinstock,

2001).

Les rats SP répondaient de façon positive aux antidépresseurs, indiquant ainsi une bonne validité prédictive de ce modèle. De plus, les antidépresseurs apparaissent comme une bonne sonde pour individualiser les mécanismes neurobiologiques qui sous-tendent les anormalités induites par le SP. L'environnement enrichi était aussi capable de reverser certaines des anormalités induites par le SP indiquant que les modifications de l'environnement post-natal tardif peuvent constituer une approche alternative valable.

Quand nous avons considéré l'efficacité des approches pharmacologiques et environnementales dans la réversion des effets induits par le stress maternel, deux questions restaient sans réponse. Premièrement, pour combien de temps ces stratégies seraient elles efficaces ? Et deuxièmement, est ce que, à un âge donné, une stratégie serait plus adaptée que l'autre ?

Une étude prospective conduite pour évaluer le fréquence et de développement des troubles mentaux, sur une cohorte suivie pendant 25 ans, a montré que la majorité des patients dépressifs souffraient d'épisodes dépressifs récurrents (Buller et Legret, 2001). En effet, dans la dépression, 50-80% des patients dépressifs rechutent lors de la phase de rémission ou développe une nouvel épisode dépressif après la guérison (Holsboer, 2001). La dépression est, pour cela, une pathologie qui nécessite souvent un traitement prolongé. De plus, un problème majeur dans le traitement de la dépression avec des antidépresseurs est représenté par le fait qu'il y a une grande incidence de rechute et de récurrence des épisodes dépressifs après l'interruption du traitement. Etant donné que les effets du SP persistent tout au long de la vie, au moins au niveau de l'axe HHS (Henry et al., 1994; Vallee et al., 1999), nous pourrions donc observer une récurrence de ces anormalités après l'interruption du traitement aux antidépresseurs car ce modèle engendre une altération constante du substrat neurobiologique.

Les antidépresseurs pourraient initier un point optimal pour un fonctionnement neuronal harmonieux. Cependant, l'interaction réciproque entre le succès du traitement et l'influence exercée par l'environnement social du patient dépressif a reçu trop peu d'attention. Dans ce contexte, un retour aux rythmes sociaux et physiques interrompus par des agents stressants psychosociaux contribuant à l'initiation et au maintien de l'état dépressif, pourrait être indirectement facilité par un traitement qui enlève un patient d'un environnement mal adapté dans lequel sa maladie perdure (Mayberg et al., 2002).

Plusieurs études conduites chez des animaux ont montré que les effets d'un environnement enrichi sur le cerveau et sur le comportement sont des effets à long terme car les augmentations de poids du cerveau et les altérations de l'activité enzymatique cholinergique ont été montrées plus de deux mois après le dernier jour d'environnement

enrichi (Diamond et al., 1976), et l'apprentissage de l'évitement de chocs électriques chez des rats adultes de 6 mois après la fin de l'enrichissement (Escorihuela et al., 1994). Cependant, la période durant laquelle l'environnement enrichi est délivré joue un rôle important dans la persistance de ces effets. Par exemple, l'enrichissement avant le sevrage n'a pas d'effets mesurables à long terme sur la neurogénèse de l'adulte (Kohl et al., 2002), ceci est en opposition à de nombreuses études qui indiquent une amélioration marquée de la neurogénèse lorsque l'enrichissement se déroule après le sevrage (Kemperman et al., 1997; Horner et Gage 2000). L'environnement enrichi exerce ses effets chez les jeunes comme chez les animaux âgés , en effet des souris âgées de 23 mois et gardées dans un environnement enrichi jusqu'à 25 mois ont montré une altération du comportement d'apprentissage (Warren et al., 1982). Ce résultat indique que le cerveau des mammifères serait encore sensible aux interventions de l'environnement lors du vieillissement.

Cette considération nous a amené à discuter du facteur âge. Les résultas sur l'efficacité et donc la validité de la pharmacologie chez les jeunes patients sont contradictoires. En fait, à la différence des adultes, les antidépresseurs tricycliques n'apparaissent pas être efficaces pour le traitement de la dépression chez les enfants et chez les adolescents (Kauffman et al., 2001). Dans ce contexte, il est important de noter que, un modèle animal de la dépression développé chez les rongeurs est produit de façon paradoxale en traitant des rats en développement , de façon chronique, entre les jours 8 et 20 suivant la naissance, avec un antidépresseur tricyclique, la clomipramine (Mirmiran et al., 1981; Vogel et al., 1990; Hansen et al., 1997; Etersen, 2002). Ces résultas indiquent que des perturbations exogènes avec des antidépresseurs, ou éventuellement d'autres psychotropes, pourrait agir pour inhiber un développement normal. Cette possibilité devait être évaluée avant de prescrire ces médicaments à de très jeunes enfants.

Avec le modèle de SP, nous avons observé tôt à l'adolescence, des anormalités comportementales, telles que une augmentation de la sensibilité aux drogues et de l'anxiété , qui s'ajoutent à celles déjà reportées au stade adulte avec l'altération de l'axe HHS. La persistance des altérations induites par le SP durant toute la vie de l'animal nous amène a penser que l'animal SP est un animal qui a manqué une étape dans son développement. Ceci nous amènerait à considérer le stade de développement de l'étape manquée. Nous pourrions suggérer que l'adolescent stressé est un individu mature trop précocement, aussi bien que l'adulte stressé est individu âgé trop précocement, car un rat SP d'âge moyen présentent des dysfonctions de l'axe HHS similaires à celles observées chez les animaux âgés (voir Vallee et al., 1999). Ce changement dans le développement représente une condition chronique qui n'est pas présente dans l'homéostasie normale c'est à dire un « état allostatique» (McEwen, 1998) qui va impliquer un *« allostatic load»,* c'est à dire le coût que

le corps aura à payer pour se forcer à s'adapter à une situation délétère (McEwen et Stellar, 1993 ; McEwen, 2001). Ces considérations mènent à suggérer que l'approche thérapeutique choisie, pour être vraiment efficace, devrait correspondre plus au stade de développement « induit par le stress » et moins au stade de développement en lui même. Ainsi, cela pourrait être intéressant d'évaluer l'existence de différences liées à l'âge chez le rat SP en réponse au traitement aux antidépresseurs qui ont été reportés précédemment, leur absence confirmerait une étape manquée dans le développement de ces animaux. .

C. DROGUES ET DEPRESSION : UN SUBSTRAT NEUROBIOLOGIQUE COMMUN?

Dans une perspective épidémiologique, il y a un haut degré de comorbidité entre la dépression et la dépendance aux drogues, ce qui indique que les taux de dépression parmi les consommateurs de drogues et les taux de consommation de drogues parmi les patients dépressifs sont plus élevés que lorsqu'un seul des deux troubles est présent (Kessler, 1996 ; pour revue, Markou et al., 1998). Cela amène à supposer que les symptômes anhédoniques de la dépression, qui constituent le cœur de cette maladie, seraient dus à un système de récompense cérébral dysfonctionnel. Ainsi, les altérations dans les processus de récompense et de motivation au niveau comportemental et neurobiologique pourraient constituer des caractéristiques définissant à la fois la dépression et la dépendance aux drogues.

Dans ce contexte, il est pertinent que le sevrage de plusieurs drogues est caractérisé par une symptomatologie dépressive (Covey et al., 1990 ; Glassman, 1993 ;). En fait, les symptômes de la phase initiale du sevrage des psychostimulants incluent dans la plupart des cas de l'anhédonie, de l'anxiété, de l'irritabilité et des troubles du sommeil (Gawin et Kleber, 1986).

Néanmoins, la relation entre ces deux troubles reste peu claire. En effet, la dépendance aux drogues et la dépression pourraient être des expressions comportementales différentes des mêmes anormalités neurobiologiques , ou un trouble psychiatrique pourrait engendrer l'autre. Une extension de ce concept faite par Markou et ses collaborateurs (1998), est connue comme l'hypothèse d'automédication et, prend en compte la possibilité que la dépendance aux drogues serait une automédication dans le but de reverser certaines des anormalités de la dépression. Il est possible que par l'utilisation simultanée de drogues multiples, les individus déterminent le drogue ou la combinaison de drogues qui normalise le mieux leur déséquilibre neurochimique qui s'exprime au niveau comportemental par la dépression. Pour ces individus, le besoin de contrôler leur symptomatologie dépressive par l'automédication jouerait un rôle important dans le maintien de la dépendance aux drogues. Par exemple, plusieurs travaux indiquent que l'administration aiguë de psychostimulants tels

que les opiacées ou l'amphétamine peut reverser temporairement les déficits neurochimiques qui pourraient être présents chez les patients dépressifs (pour revue, Markou et al., 1998; Tremblay et al., 2002). Aucune de ces consommations de drogue ne sont cependant considérées comme des antidépresseurs cliniquement efficaces par les praticiens (Kaufman et al., 1984 ; Naranjo et al., 2001). En tout cas, la possibilité demeure que l'utilisation simultanée ou séquentielle de drogues variées comme auto prescrites par les besoins émotionnels des consommateurs de drogues, mène à un effet antidépresseur adéquat, mais dans le même temps, entraîne les individus dans un état actif de dépendance aux drogues. Le meilleur support clinique pour cette hypothèse de l'automédication est fourni par le fait que le traitement aux antidépresseurs est significativement plus efficace en réduisant la consommation de drogue chez les dépressifs consommateurs de drogue que chez les consommateurs non dépressifs (Kleber et al., 1983; Ziedonis et Kosten, 1991; Nunes et al., 1993 ; 1995). Indépendamment du fait que la dépression soit présente avant l'abus de drogues ou que la dépression soit induite par la drogue, la diminution de la consommation de drogue observée avec les antidépresseurs suggère que, lorsqu'il y a un allégement de la symptomatologie par la consommation d'antidépresseurs , le besoin d'automédication de drogues diminue (Markou et al., 1998).

Ainsi, plusieurs aspects suggèrent que ces deux troubles psychiatriques pourrait être liés par une neurobiologie commune. Dans ce contexte, la sérotonine apparaît comme impliquée de façon critique dans la dépression (Meltzer et Lowy, 1987; Briley et Moret, 1993 ; Blier et de Montigny, 1994), mais les altérations de la neurotransmission sérotoninergique sont aussi un des changements se produisant lors du sevrage aux psychostimulants (Parsons et al., 1995; Naranjo et al., 2001; Harrison et Markou, 2001), de plus, un traitement chronique aux ISRS est efficace dans la réversion des aspects affectifs du sevrage aux drogues (Harrison et al., 2001). Lorsque l'on considère un autre aspect commun à la dépression et à la dépendance aux drogues, de récents travaux ont montré qu'une altération de la plasticité cérébrale était une conséquence de ces troubles psychiatriques. Dans la dépression, le déclin et l'augmentation de la neurogénèse sont des facteurs importants, respectivement dans son origine et dans sa rémission (Eisch, 2002 ; Jacobs et al. 2002 ; Nestler et al., 2002 ; McEwen, 2001). Les études conduites chez l'animal ont montré qu'un traitement répété à l'amphétamine ou à la cocaïne est capable d'altérer la structure des dendrites dans le noyau accumbens et dans le cortex préfrontal (Robinson et Kolb, 1997 ; 1999) et, une diminution dans la neurogénèse est observée après l'auto-administration de morphine (Eisch et al., 2000) ou de nicotine (Abrous et al., 2002). Dans ce contexte, ces résultats supportent l'existence de substrats communs sous-tendant les effets persistants sur les circuits neuronaux impliqués dans les troubles dépressifs et dans la consommation de drogues.

Chez le rat SP, on a montré une diminution de la neurogénèse (Lemaire et al., 2000), des

dysfonctions du système de récompense (Fride et Weinstock, 1988; Takahashi et al., 1992; Henry et al., 1995), et des anormalités sérotoninergiques (Peters, 1988 ; 1990 ; article n.5 de cette thèse) et opioïdergiques (Weinstock et al., 2001). De plus, nous avons observé une augmentation de la réponse aux antidépresseurs (articles n 4 et 5) et aux psychostimulants (article n3, Deminiere et al., 1992 ; Koel et al., 2000). Ainsi, le modèle de SP chez le rat devrait être considéré comme un bon modèle pour l'investigation de pathologies multiples corrélées. Comme ces effets sont persistants durant toute la vie de l'animal (Henry et al., 1994; Vallee et al., 1999; Lemaire et al., 2000) il représente aussi un modèle animal plus avantageux que les autres qui présentent le même profil de maladies coexistantes (stress chronique léger) , mais n'ont que des effets transitoires (effets observables 2 mois après la fin du paradigme stressant).

Globalement, l'existence de comorbidité entre la dépression et l'abus de drogues souligne l'importance de l'adoption d'une approche intégrée dans le traitement de ces troubles, ou le système cérébral de récompense pourrait être considéré comme une cible thérapeutique importante (Naranjo et al., 2001). La compréhension des mécanismes neurobiologiques et comportementales médiant cette comorbidité pourrait conduire non seulement au développement de meilleurs traitements mais augmenterait également notre compréhension des mécanismes influençant les processus motivationnels et affectifs à la fois chez les sujets sains et chez les patients. Finalement, en considérant que tous les troubles psychiatriques incluant la dépression et la dépendance aux drogues, impliquent des symptômes comportementaux qui reflètent des anormalités neurobiologiques, le progrès dans lke compréhension de ces pathologies à plusieurs niveaux d'analyse pourrait certainement impliquer une approche de recherche multidisciplinaire. Dans ce contexte, il serait intéressant d'explorer la dépression par un modèle animal de la dépendance aux drogues et vice versa. De plus, l'exploration de l'hypothèse de l'automédication testerait les effets de la consommation de drogues variées sur la réversion de la symptomatologie dans des modèles animaux de la dépression.

Cette thèse avait pour but d'évaluer la validité de deux modèles animaux qui modulent le degré de la différentiation sexuelle (le modèle de la PIU chez la souris) et l'activité de l'axe HHS (le modèle du rat SP), respectivement, afin d'étudier l'augmentation de la vulnérabilité aux psychostimulants et aux troubles dépressifs. Nous avons montré que les deux modèles exercent des effets à long terme sur les stratégies d'adaptation d'un individu aux conditions environnementales. Nous avons également montré que deux stratégies d'intervention étaient capables de réduire les altérations comportementales et neurobiologiques induites par le stress maternel.

Les études conduites dans cette thèse montrent l'importance de considérer l'impact de

perturbations subtiles et / ou intenses dans l'environnement précoce d'un individu en développement, comme les facteurs programmateurs qui peuvent réguler l'expression de ses futures capacités adaptatrices. De plus, ces études soulignent la nécessité d'adopter une approche intégrée dans l 'évaluation des interventions thérapeutiques.

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