Pergamon

PII: S0306-4522(00)00336-5

www.elsevier.com/locate/neuroscience

Neuroscience Vol. 100, No. 4, pp. 749–768, 2000

0306-4522/00 $20.00+0.00

BEHAVIORAL, NEUROCHEMICAL AND ENDOCRINOLOGICAL CHARACTERIZATION OF THE EARLY SOCIAL ISOLATION SYNDROME

C. A. HEIDBREDER,*† I. C. WEISS,‡ A. M. DOMENEY,‡ C. PRYCE,‡ J. HOMBERG,‡ G. HEDOU,‡ J. FELDON,‡ M. C. MORAN§ and P. NELSON§

*SmithKline Beecham Pharmaceuticals, Department of Neuroscience, New Frontiers Science Park (North), Building H25, Room 104A, Harlow, Essex CM19 5AW, UK

‡The Swiss Federal Institute of Technology Zürich (ETH), Laboratory of Behavioral Biology, Zürich, Switzerland

§SmithKline Beecham Pharmaceuticals, Department of Statistical Sciences, Harlow, Essex, UK

Abstract—Rearing rats in isolation has been shown to be a relevant paradigm for studying early life stress and understanding the genesis of depression and related affective disorders. Recent studies from our laboratory point to the relevance of studying the social isolation syndrome as a function of home caging conditions. Accordingly, the present series of experiments assessed the contribution of each condition to the expression of the prepulse inhibition of the acoustic startle, food hoarding and spontaneous locomotor activity. In addition, *ex vivo* neurochemical changes in the brains of isolated and grouped rats reared either in sawdust-lined or in grid-floor cages were determined by measuring dopamine and serotonin as well as their major metabolites in a "psychosis circuit" that includes mainly the hippocampus and selected hippocampal efferent pathways projecting towards the anterior cingulate and infralimbic cortices, nucleus accumbens, dorsolateral caudate nucleus, amygdala and entorhinal cortex. The results of the present study demonstrate that rearing rats in isolation (i) produces a syndrome of generalized locomotor hyperactivity; (ii) increases the startle response; (iii) impairs prepulse inhibition; (iv) tends to increase food hoarding behavior; (v) increases basal dopamine turnover in the amygdaloid complex; (vi) decreases basal dopamine turnover in the infralimbic part of the medial prefrontal cortex; and (vii) decreases basal turnover of serotonin in the nucleus accumbens. In the entorhinal cortex, dopamine neurotransmission seemed to be more sensitive to the caging conditions since a decreased basal turnover of dopamine was observed in grid-reared animals. Plasma corticosterone levels were also increased in grid-reared animals compared with rats reared in sawdust cages. Finally, isolates reared on grids showed a significant positive correlation between plasma corticosterone levels and dopamine in the left nucleus accumbens.

Altogether, these results support the contention that there is a link between social isolation, attention deficit, spontaneous locomotor hyperactivity and reduced dopamine turnover in the medial prefrontal cortex. Furthermore, our data demonstrate that rearing rats in grid-floor cages represents a form of chronic mild stress associated with increased corticosterone levels, decreased basal turnover of entorhinal dopamine and increased dopamine activity in the left nucleus accumbens. Finally, a significant and selective decrease in the basal turnover of serotonin in the nucleus accumbens of isolated rats may be linked to the isolation-induced locomotor hyperactivity. © 2000 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: caging conditions, dopamine, interhemispheric coupling, principal components analysis, serotonin, social isolation.

Events experienced in early life may contribute to the expression or exacerbation of a variety of physical and psychological disorders.[2] Rearing rats in isolation has been shown to be a relevant paradigm for studying early life stress and understanding the genesis of depression and related affective disorders.[18] The so-called isolation syndrome has been well characterized and consists of spontaneous and conditioned locomotor hyperactivity,[17,27–29,68,69,85] enhanced responses to novel environments,[27–29,67] greater tendencies towards perseveration,[19,43,54] deficits in prepulse inhibition (PPI)[17,30,84–86] and altered responses to the behavioral effects of drugs such as barbiturates,[20,44] opioids,[46] neuroleptic drugs like α-flupenthixol[66] and dopamine agonists such as amphetamine-like psychostimulants.[42,43,60,67,72,74,85,87] We have also demonstrated recently that the social isolation syndrome differs as a function of home caging condition.[85] Specifically, isolates reared in sawdust cages showed a significant deficit of PPI, which was not apparent in rats reared in grid-floor cages. Furthermore, group-housed animals reared in grid-floor cages showed a significant PPI deficit compared with their sawdust-reared counterparts. Finally, animals housed in grid-floor cages showed reduced spontaneous locomotor activity compared with sawdust-caged animals. Thus, these results suggest that, in addition to the social isolation *per se*, the caging conditions may interfere with spontaneous locomotion and normal sensorimotor gating mechanisms.

The behavioral hyperactivity as well as the increased responsiveness to dopamine agonists that both develop following isolation suggest that rearing rats in isolation affects central dopaminergic mechanisms. Consistent with this hypothesis, *in vivo* microdialysis experiments reveal that isolation-reared rats exhibit higher dialysate dopamine levels in both the dorsal striatum and nucleus accumbens (NAC) in response to the systemic administration of 2 mg/kg *d*-amphetamine.[43] However, *ex vivo* measurements of dopamine show that isolation-reared animals have higher *post mortem*

†To whom correspondence should be addressed. Tel.: +44-1279-622457; fax: +44-1279-622660.

E-mail address: Christian_A_Heidbreder@sbphrd.com (C. A. Heidbreder).

Abbreviations: ACTH, adrenocorticotrophic hormone; ANOVA, analysis of variance; CING, anterior cingulate cortex; CP, dorsolateral caudate nucleus; DOPAC, 3,4-dihydroxyphenylacetic acid; EC, entorhinal cortex; EDTA, ethylenediaminetetra-acetate; 5-HT, 5-hydroxytryptamine; HPLC, high-performance liquid chromatography; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; INFRA, infralimbic cortex; NAC, nucleus accumbens; mPFC, medial prefrontal cortex; PCA, principal components analysis; PLS–DA, partial least squares–discriminant analysis; PPI, prepulse inhibition.

dopamine concentrations in the medial prefrontal cortex (mPFC) but not in subcortical areas.[43] Recent studies also demonstrate that exposure to footshock and conditioning to context produce increases in extracellular serotonin (5-hydroxytryptamine; 5-HT) in rats reared in social isolation from weaning,[24] suggesting that the previously reported enhancement in presynaptic dopamine function within the NAC is associated with enhanced serotonergic functions within the mesolimbic system. Isolation rearing is also associated with increased responsiveness of postsynaptic 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors.[22,88]

In the present series of experiments, behavioral, neuroendocrine and neurochemical alterations in both isolated and grouped rats reared either in sawdust-lined or in grid-floor cages were investigated. Behavioral studies were designed to compare, within the same experiment, the effects of social isolation in animals reared in different caging conditions. Both caging conditions were established and tested at the same time, in order to evaluate the contribution of each condition to the expression of PPI and two additional behavioral paradigms known to be sensitive to dysfunction of the mPFC: food hoarding and spontaneous locomotor activity. The *ex vivo* neurochemical changes in the brains of isolated and grouped rats reared either in sawdust-lined or in grid-floor cages were investigated by measuring biogenic monoamines in the two main cytoarchitectural subdivisions of the mPFC, the anterior cingulate (CING) and infralimbic (INFRA) cortices, as well as the NAC, and dorsolateral caudate nucleus (CP) from both the left and right hemispheres. Biogenic monoamines in the left and right amygdala and hippocampus were also analysed since dopamine modulates the excitatory responses of NAC neurons to stimulation of these regions[53,90,91] and since alterations in catecholamine function in both the amygdala and hippocampus have been reported following social isolation in rats.[79] In fact, under normal conditions, the mPFC would provide goal-directed motor plans that are selected within the NAC on the basis of information originating from both the hippocampus (contextual constraints) and amygdala (emotion or affective state).[31] It is hypothesized that pathological dysfunction within this corticoaccumbens loop would cause the system to respond inappropriately to otherwise insignificant stimuli. Specifically, failure of the hippocampus and amygdala in gating mPFC throughput at the level of the NAC would bias the corticoaccumbens system to react exclusively on the basis of the affective valence of stimuli and would result in an inability to use goals or contextual cues to guide behavior. *Post mortem* measurements were also performed in the left and right entorhinal cortex (EC), which has been implicated in a "psychosis circuit"[78] that includes mainly the hippocampus and selected hippocampal efferent pathways projecting towards the CING, the subiculum of the hippocampus, the anteroventral thalamus and the EC. Since different patterns of cerebral lateralization may be related to an alteration in interhemispheric communication,[5,35,89] we also assessed interhemispheric coupling by determining correlation coefficients between neurotransmitters in the left and right hemispheres.[13,14] Finally, given that both social isolation and caging condition have been implicated as chronic stressors and, furthermore, glucocorticoids are known to regulate dopamine transmission, plasma levels of both adrenocorticotrophic hormone (ACTH) and corticosterone of isolation-reared rats in each caging condition (sawdust vs grid-floor) were compared with rats reared in social groups in similar caging conditions.

EXPERIMENTAL PROCEDURES

Subjects

The studies used male Wistar rats (bred at the Laboratory of Behavioral Biology, Schwerzenbach). Animals were housed under standard conditions in a temperature ($21 \pm 1°C$) and humidity ($55 \pm 5\%$) controlled room. Animals received food (Nafag, 9431, Nafag Ecosan, Gossau, Switzerland) and water *ad libitum*. The light schedule in the room was reversed, with lights on between 19:00 and 07:00, and all experiments were conducted between 09:00 and 18:00. All experiments were carried out in agreement with the Swiss Federal Regulations for animal experimentation.

Experimental design

Four groups of animals were used in the present study. The animals were randomly separated at weaning (21 days) into two different rearing conditions: isolation-rearing vs group-housed rearing. These groups were then divided into two caging conditions: animals reared in grid-floor cages vs animals reared in sawdust cages. Thus, two groups (isolates and grouped) were housed in Macrolon cages containing wire grid-floors (dimensions: $48 \times 27 \times 20$ cm for isolates vs $59 \times 38.5 \times 20$ cm for group-housed, $n = 4$ rats per cage), with a plastic underhanging tray for the collection of urine and faeces. Animals were only disturbed for cleaning purposes, which consisted of changing the tray twice a week for both isolates and grouped animals. At no time were animals handled during the period before experimental manipulations commenced. The other two groups (isolates and grouped) were housed in solid-bottom cages of identical dimensions to those described above, but containing sawdust. The animals reared in sawdust cages were disturbed only once a week for cage cleaning, whereas the grouped animals reared in sawdust cages were cleaned twice a week. The experimental testing commenced 12 weeks after the appropriate housing condition.

Open field spontaneous locomotor activity

The apparatus consisted of four square arenas ($76.5 \times 76.5 \times 49$ cm) made of dark grey plastic, which were located in an experimental room illuminated by low light (12 lux). A video camera fixed above the four arenas and relayed to a monitor and a video tracking motion analysis system (Ethovision, Noldus Information Technology bv, Wageningen, The Netherlands) allowed the recording of the locomotor activity of the rats (total distance travelled in the entire arena or in the centre in centimetres). After 1 h habituation to the test room, each rat was individually placed into the centre of the open field arena. The animals were tested, in counterbalanced groups of four, for a 60 min test session. The locomotor activity was measured in bins of 10 min.

Prepulse inhibition

PPI was assessed in four sound attenuated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), which were illuminated and ventilated. Each startle chamber consisted of a stabilimeter system composed of a transparent Plexiglas cylinder (Ø 8.2 cm, length 20 cm) mounted on a Plexiglas frame. A speaker mounted 24 cm above the cylinder provided the acoustic noise bursts. The startle responses of the rat within the cylinder were detected and transduced by a piezoelectric accelerometer mounted below the frame. Startle amplitudes were defined as the average of 100 1-ms stabilimeter readings collected from the stimulus onset. Before each test session, the stabilimeter system was calibrated. The startle session started with a 5 min acclimatization period, with a 68 dB[A] background noise level that continued throughout the test session. To evaluate the basal startle response, four startle pulses of 120 dB[A], 30 ms duration were then presented to the animal. Next, the animal received six blocks of 11 trials to measure PPI. Each block consisted of four different trial types, presented pseudo-randomly throughout the session, i.e. pulse alone (two trials), prepulse alone (one trial for each prepulse intensity), prepulse followed by pulse (one trial for each prepulse intensity) or no stimulus (one trial). The four different prepulses had an intensity of either 72, 76, 80 or 84 dB[A] and a duration of 20 ms. The time interval between the prepulse offset and the pulse onset was 80 ms. The

percentage of PPI induced by each prepulse intensity was calculated as: [100 − (100 × Startle amplitude on prepulse trial)/(Startle amplitude on pulse alone trial)].

Food hoarding

Prior to the food hoarding test, animals were progressively food deprived during six days to reach 85% of their initial body weight. During this period, food pellets (approximately 15 g) were given regularly to the rats every day at 12:00. The food hoarding system consisted of two Macrolon cages type III (dimensions 48.0 × 27.0 × 20.0 cm) with drinking and feeding devices in the front. One of these two cages had sawdust-bedding material and was used as the "home cage". The other cage did not have any sawdust and was used as the hoarding cage. The two cages were connected together so that the feeding device of the home cage provided an entrance to the hoarding cage. The entrance could be closed by a piece of Plexiglas. Animals were randomly and equally subdivided to be tested in the food hoarding system. The test room was dimly illuminated (2 × 60 W indirect). On the test day, rats were tested in squads of seven. Fifty food pellets were weighed before being distributed in the hoarding cage. The animals were all placed in the home cage, with the entrance closed by the Plexiglas door. A habituation to this home cage was allowed during 30 min. Thereafter, the entrance to the hoarding cage was opened, so that rats had free access to the entire system. The test duration was 1 h, after which time the entrance of the hoarding cage was closed, and all the animals were put back in the animal room. The pellets in the hoarding cage and in the home cage were counted and weighed. Two hoarding scores were calculated: the total weight and the number of food pellets removed from the hoarding cage and carried into the home cage.

Brain tissue sample preparation

After completion of the behavioral experiments, rats were killed by decapitation and the brains rapidly removed, quickly frozen and stored at −80°C until assay. Frozen brains were placed ventral side up in a rat brain matrice (Harvard Apparatus, South Natick, MA, USA) onto an ice-chilled plate. Three double-edge blades were used to prepare coronal sections (1500–1800 μm thick). The first blade was placed in the matrice's channel lying on the caudalmost boundary of the slice that was planned to be taken; the two other blades were then located in the channels that corresponded to the rostral boundaries. Once all three blades were in place, the first and second blades were lifted out to take the desired slice while leaving the third blade in place. The partially frozen slices were placed on an ice-cold dissection plate for removal of discrete brain regions using a stereomicroscope and a micropunch system (MP-600, Harvard Apparatus, South Natick, MA, USA). Punch tips (∅ 1, 2 mm) were pushed into the region of interest and then withdrawn. A gas air supply was connected to the micropunch system at a pressure of 40 psi. The output pressure used to eject the tissue punch from the cutting punch tip was adjusted to 12 psi. Tissue punches from both the left and right hemispheres were taken for subsequent neurochemical assays. Using the notch of the optic chiasm as an external landmark, coronal cuts were made to separate each brain into four slices according to the atlas of Palkovits and Brownstein.[58] A first slice (A5400 to A3900 μm) was prepared to punch out the frontopolar cortex with its medial (rostral cingulate) cortex and ventral (prelimbic/infralimbic) cortex (Fig. 1A). A second slice (A3000 to A1200 μm) was used to punch out the NAC and the dorsolateral part of the head of the caudate nucleus, lateral to the ventricle and otherwise enveloped by the corpus callosum laterally and dorsally (Fig. 1B). A third slice (P2100 to P3800 μm) was used to punch out the superior or dorsal hippocampus and the rostral portion of the dentate gyrus. The same slice was used to punch out the lateral amygdaloid nuclei bounded laterally by the external capsule, and surrounded by the ventral portion of the caudate–putamen and the central amygdaloid nucleus. The same punching also included the central, medial, medial posterior, basal and basal lateral nuclei, but excluded the most ventral nucleus of the amygdala (cortical amygdaloid nucleus), the posterior amygdaloid nucleus in the caudal third of the amygdaloid complex, and the amygdalohippocampal area (Fig. 2A). Finally, a fourth slice (P4200 to P5700 μm) was used to punch out the entorhinal cortex occupying the ventrolateral part of each hemisphere (Fig. 2B).

Tissue samples were weighed and the same area on each side of the brain was placed in a 1.5 ml polypropylene microcentrifuge tube and homogenized using a tapered motorized pestle in 300 μl of ice-cold mobile phase. After centrifugation at 20,000 g for 15 min at 4°C, the clear supernatant layers were removed into a 1 ml syringe and filtered through a 0.45 μm Nylon (Iso-Disk N-34, 3 mm) filter to separate the insoluble residue. A portion of the supernatant was carefully transferred to a small tube and further centrifuged at 20,000 g for 2 min. An aliquot (30 μl) of the supernatant was then injected onto the high-performance liquid chromatography (HPLC) system for the assessment of monoamines.

Chromatographic conditions

A chromatography workstation (Millenium, Millipore Corp., Bedford, MA) was used in conjunction with solvent delivery pumps (Waters 515 HPLC Pump, Milford, USA) and electrochemical amperometric detectors (Antec-Decade, Leyden, The Netherlands). A six-port rotary valve (Model 7125, Rheodyne, Berkeley, CA, USA) was used for sample injection. All chemicals and analytical grade reagents were obtained from Sigma Chemical Co. (St Louis, MO, USA) and Fluka BioChemica (Ronkonkoma, NY, USA). A glassy carbon working electrode was used at a voltage setting of +750 mV versus an Ag/AgCl reference electrode for the detection of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT and 5-hydroxyindoleacetic (5-HIAA). Chromatographic separations were performed using a Chrompack glass column [100 (L) × 3 (ID∅) × 9 (OD∅) mm] packed on microparticulate (5 μm) silica gel. The mobile phase consisted of 0.15 M $NaH_2PO_4.H_2O$, 1.6 mM sodium octanesulfonate, 1.0 mM EDTA and 10% methanol (v/v), adjusted to pH 4.3. The mobile phase was filtered through a 0.22 μm filter (Millipore Corp., Bedford, MA, USA), degassed under vacuum and delivered at a flow rate of 1.0 ml/min. The position and height of the peaks in tissue homogenates were compared with 30 μl samples of an external calibrating standard solution containing 100, 10 and 1 nM. The detection limit for dopamine and 5-HT was 1.0 fmol. To compensate for gradual decay in detector sensitivity during a working day, 30 μl of working standard solutions was injected into the HPLC system after every fifth tissue sample. The levels of substrates in each brain sample were expressed as nanograms per gram of wet tissue weight.

Adrenocorticotrophic hormone and corticosterone radioimmunoassay

Following decapitation (same subjects as for the behavioral studies and monoamine assays), trunk blood samples were collected into prechilled EDTA-coated tubes (Sarstedt AG, Sevelen, Switzerland), centrifuged, and the plasma stored at −80°C prior to measurement of ACTH and corticosterone immunoreactivity. ACTH titres were determined within a single assay using an ^{125}I radioimmunoassay kit for human ACTH (DiaSorin, Stillwater, MN, USA) which we validated for the rat in terms of accuracy and parallelism. Rabbit anti-ACTH serum was incubated with ^{125}I-ACTH and either ACTH standard (porcine, 2–50 pg/100 μl/tube, in triplicate) or sample (50 μl/tube, in duplicate), and separation was achieved using a precipitating complex of goat anti-rabbit globulin and polyethylene glycol. Intra-assay precision was 5% ($n = 6$). Corticosterone titres were determined using an in-house ^{3}H radioimmunoassay validated for rat EDTA plasma. Diluted plasma samples were heated in a water bath at 90°C for binding protein denaturation, and rabbit anti-corticosterone serum (07-120016, ICN Biomedicals Inc, Costa Mesa, CA, USA) was incubated with [1,2,6,7-^{3}H] corticosterone (TRK 406, Amersham Switzerland, Zürich-CH) and either corticosterone standard (Sigma, C-2505, 12.5–250 pg/250 μl/tube, in triplicate) or sample (250 μl at 1:400 dilution, in duplicate); separation was achieved using dextran-coated charcoal. All samples were determined within a single assay and intra-assay precision was 3%.

Data analysis

The effect of rearing and caging conditions on locomotor activity was analysed using a three-way analysis of variance (ANOVA) with two between-subjects factors of rearing condition (isolates vs group-housed) and caging condition (sawdust vs grids), and a repeated measurements factor of time (six blocks of 10 min each). The amplitude of the startle response was analysed using an overall three-way ANOVA with two between-subjects factors of rearing condition (isolates vs group-housed) and caging condition (sawdust vs grids), and a repeated measurements factor of 16 pulse-alone presentations.

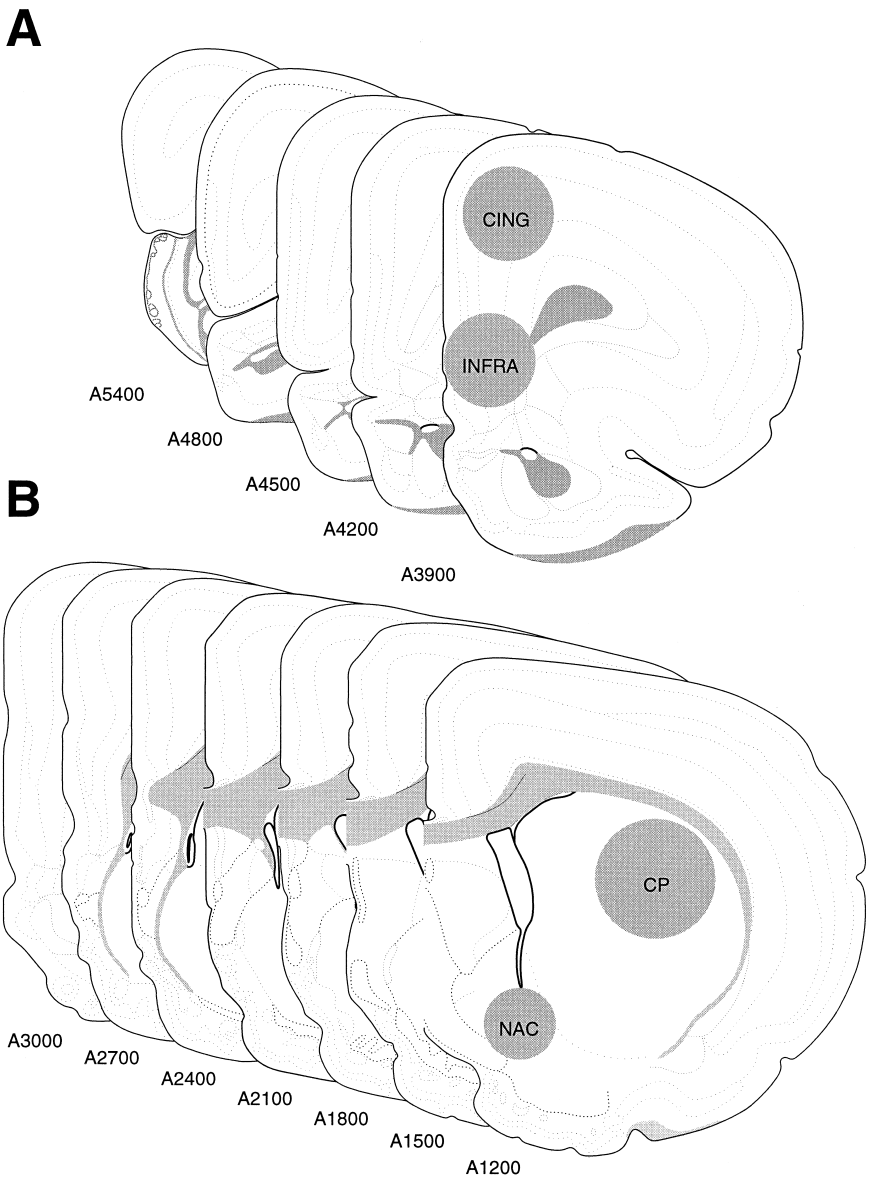

Fig. 1. Silhouette drawings of micropunchings onto representative sections of the anterior part of the rat brain.[77](A) Depicts two tissue micropunchings on a 1500 μm slice preparation (A5400 to A3900 μm according to Palkovits and Brownstein):[57] anterior cingulate cortex (CING) and infralimbic cortex (INFRA). (B) Represents two tissue micropunchings on a 1800 μm slice preparation (A3000 to A1200 μm): nucleus accumbens (NAC) and dorsolateral part of the caudate–putamen.

The mean percentage PPI was analysed by a 2×4 ANOVA with between-subjects factors of rearing condition (isolates vs group-housed) and caging condition (sawdust vs grids), and a repeated measurements factor of the four prepulse intensities (72, 76, 80 and 84 dB[A], respectively). Concerning the food hoarding behavior, additional ANOVAs consisting of two between-subjects factors of rearing and caging condition were conducted on the number and weight of pellets hoarded as well as on the number of pellets eaten by the rat.

In the case of each neurotransmitter an initial ANOVA with rearing (isolation vs group housing) and caging (grid-floor vs sawdust) conditions as between-subjects factors and hemispheres (left vs right) as a within-subjects factor was performed to gain information about the important effects involved. Means graphs involving all four factors were plotted as a visual aid to the ANOVA output. Grouped animals reared on sawdust were used as the control group for all comparisons involving rearing and caging conditions. Where variables have been log transformed and subsequently 95% confidence intervals for the differences between subgroups have been calculated, the confidence intervals are ratios of the difference. Any such confidence interval for a ratio that does not include one can be regarded as a statistically significant difference. Where response variables have not needed to be log transformed, confidence intervals for a difference not including zero can be considered statistically significant. When confidence intervals have been calculated taking into account the factor hemisphere, the control is the grouped animals reared on sawdust in the same hemisphere as the subgroup with which it is being compared.

One of the major problems with the generation of a great many measurements is one of locating and extracting the relevant neurochemical information from so much data and interpreting it easily and reliably. The information about the effects of rearing and caging conditions on dopaminergic and serotonergic function is unlikely to lie in any isolated neurochemical, but rather in a combination of several neurochemicals. The solution is to couple univariate analyses with multivariate data analysis, such as principal components analysis (PCA) and partial least squares (PLS) to determine key variables and to simplify both the analysis and visualization of multidimensional data sets. By analysing all of the neurochemical measurements simultaneously, multivariate methods utilize the correlations between the variables to efficiently compress the high dimensional coordinate system to one comprising only a few relevant axes that describe major patterns and trends in the data. The information can then be displayed in simple graphs for easy, but comprehensive interpretation. For more information on data compression and

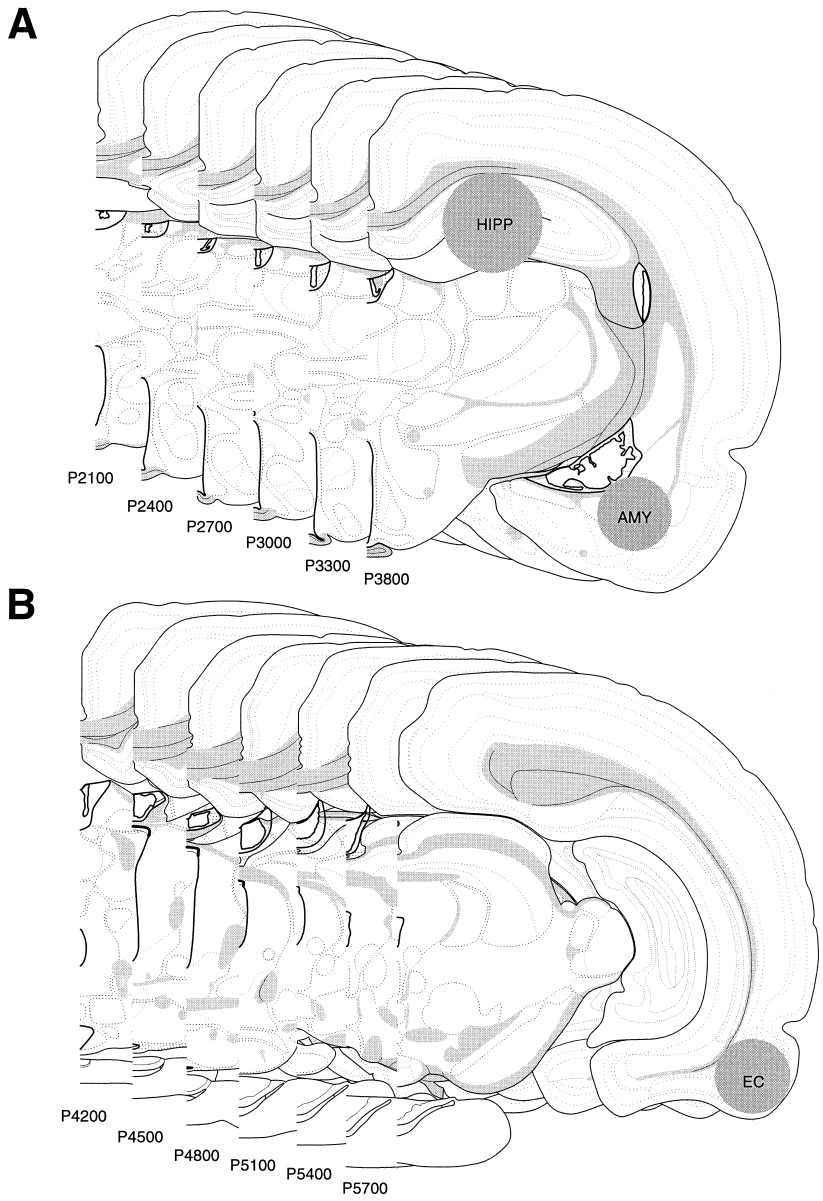

Fig. 2. Silhouette drawings of micropunchings onto representative sections of the posterior part of the rat brain.[77](A) Depicts two tissue micropunchings on a 1700 μm slice preparation (P2100 to P3800 μm according to Palkovits and Brownstein):[57] dorsal hippocampus and amygdaloid complex. (B) Represents one tissue micropunching on a 1500 μm slice preparation (P4200 to P5700 μm): EC.

information extraction using principal components, please refer to Ref. 40.

The effect of rearing and caging conditions on plasma levels of corticosterone and ACTH was analysed by a two-way ANOVA with main factors of rearing (isolation vs group housing) and caging (grid-floor vs sawdust) conditions. In the case of significant main effects, the differences between individual means were assessed with the post hoc Fisher's Protected LSD test. The relationship between plasma corticosterone levels and both dopamine and 5-HT in the amygdala, EC, hippocampus, CING, INFRA, CP and NAC was analysed using regression analyses. The determination rates of the regression model, as well as the partial adjusted regression coefficients, their tests of significance and *P*-values for every variable were calculated. Statistical significance was set at a probability level of $P < 0.05$ for all tests. Finally, interhemispheric coupling was assessed by determining correlation coefficients between absolute amounts of dopamine in the left and right hemispheres.[13,14] For each particular combination of brain region and neurotransmitter, a Pearson correlation matrix of *r* coefficients was computed between the left and right hemispheres for each rearing × caging group of rats. Significant *P*-levels were determined at the 5% level of confidence. The strength of left–right correlations was then assessed using the weighted Fisher's *z* coefficients.[13]

RESULTS

Effect of isolation rearing and home caging conditions on locomotor activity

All of the animals showed a significant habituation to the open field environment, as reflected by a gradual decrease in their locomotor activity (expressed in total distance travelled) throughout the test session ($F_{5,140} = 95.2$, $P < 0.0001$). There was statistical evidence for different profiles of locomotor activity in the entire open field after social isolation or caging on grid-floors. Rats reared in isolation showed higher locomotor activity scores than their grouped congeners, mainly for the first 40 min of the session. The overall ANOVA yielded a significant effect of rearing condition ($F_{1,28} = 12.7$, $P < 0.001$) and a significant rearing × time bins interaction ($F_{5,140} = 5.1$, $P < 0.001$; see Fig. 3A). Moreover, animals reared on grid-floors showed reduced locomotor activity relative to rats reared on sawdust, especially for the first 10 min of

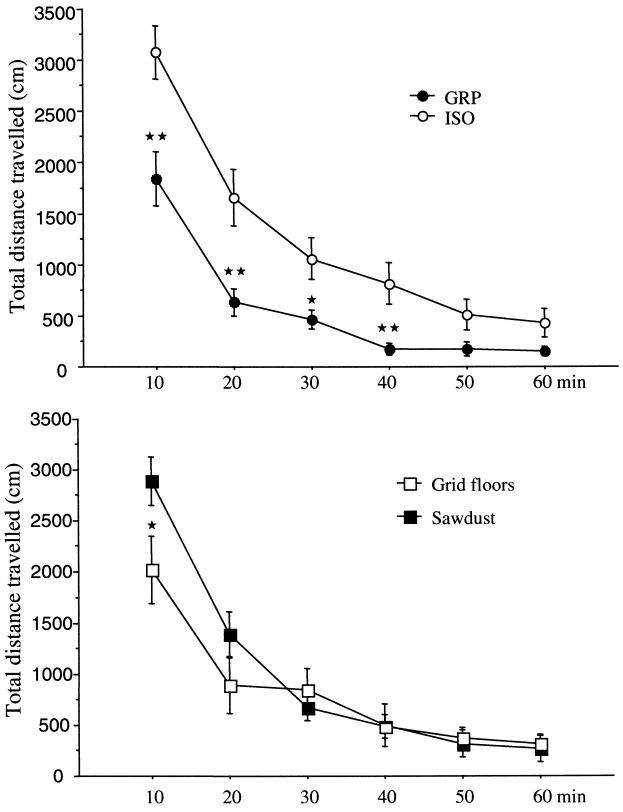

Fig. 3. Effect of rearing and caging conditions on spontaneous locomotor activity in the open field. Locomotor activity (mean ± S.E.M.) is represented as the total distance travelled (cm) by rats reared either in isolation (ISOL) or in groups (GRP) (upper panel, data collapsed over caging conditions) and by rats reared either on sawdust or on grid-floor cages (lower panel, data collapsed over rearing conditions). $*P < 0.05$; $**P < 0.01$ vs corresponding group.

the session (caging × time bins interaction, $F_{5,140} = 5.4$, $P < 0.001$; see Fig. 3B). There was no main effect of caging condition and no caging × rearing interaction for the locomotor activity in the entire arena ($F_{1,28} = 1.2$, $P = 0.3$ and $F_{1,28} = 0.4$, $P = 0.5$, respectively).

The animals also reduced gradually their locomotor activity in the centre of the arena throughout the test session ($F_{5,140} = 17.5$, $P < 0.0001$). In addition, as observed for the locomotor activity in the entire arena, isolated rats showed higher levels of activity in the centre of the arena than grouped animals. The overall ANOVA yielded a significant effect of rearing condition ($F_{1,28} = 6.04$, $P < 0.05$) and a significant rearing × time bins interaction ($F_{5,140} = 3.0$, $P < 0.05$). Furthermore, rats reared on grid-floors showed decreased activity in the centre relative to animals reared on sawdust (caging × time bins interaction, $F_{5,140} = 7.0$, $P < 0.001$). Finally, there was no main effect of caging condition and no caging × rearing interaction for the locomotor activity in the entire arena ($F_{1,28} = 2.4$, $P = 0.1$ and $F_{1,28} = 0.08$, $P = 0.8$, respectively).

Effect of isolation rearing and home caging conditions on startle response and prepulse inhibition

A trend towards increased startle response in isolates (1274.2 ± 72.5) compared with grouped controls (1065.0 ± 74.1) was apparent ($F_{1,28} = 4.1$, $P = 0.05$), whereas caging conditions did not affect the startle response

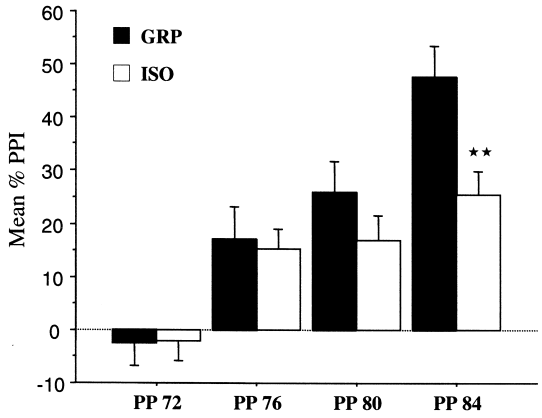

Fig. 4. Effect of isolation rearing on the PPI response. Means ± S.E.M. of percentage PPI of group-housed and isolates are presented for each prepulse intensity tested (72, 76, 80 or 84 dB[A]). $**P < 0.01$ vs group-housed animals.

($F_{1,28} = 1.8$, $P = 0.2$). There was no caging × rearing interaction for the startle response ($F_{1,28} = 0.05$, $P = 0.8$). All animals showed a gradual reduction in their startle responses throughout the 16 pulse alone presentations ($F_{15,420} = 7.1$, $P < 0.0001$), which was similar for the four experimental groups.

There was no statistical evidence of a main effect of either caging or rearing conditions on the mean PPI response ($F_{1,28} = 0.09$, $P = 0.8$ and $F_{1,28} = 2.08$, $P = 0.2$, respectively). However, a rearing × prepulse intensities interaction ($F_{3,84} = 4.4$, $P < 0.01$) revealed a significant isolation-induced PPI deficit at the prepulse intensity of 84 dB[A] (see Fig. 4). Finally, there was a significant effect of prepulse intensity ($F_{3,84} = 44.0$, $P < 0.0001$), reflecting the increased effectiveness of higher prepulse intensities in inducing stronger PPI.

Effect of isolation rearing and home caging conditions on food hoarding behavior

There was only weak evidence of a statistically significant effect of both caging and rearing conditions on the number of pellets hoarded. This suggested an additive effect of isolation and sawdust floors on increasing the number of pellets hoarded by rats on average. An overall ANOVA with main factors of rearing and caging conditions revealed an almost significant effect of rearing condition ($F_{1,28} = 3.2$, $P = 0.084$) and caging condition ($F_{1,28} = 3.1$, $P = 0.088$), but no significant rearing × caging interaction ($F_{1,28} = 0.007$, $P = 0.9$). Similar to the number of pellets hoarded, there was only weak evidence of statistically significant effects due to both rearing and caging conditions on the weight of pellets hoarded, again suggesting an additive effect of isolation and sawdust floors on increasing the weight of pellets hoarded by rats on average. An overall ANOVA with main factors of rearing and caging conditions revealed an almost significant effect of rearing condition ($F_{1,28} = 3.2$, $P = 0.086$) and caging condition ($F_{1,28} = 3.2$, $P = 0.086$), but no significant rearing × caging interaction ($F_{1,28} = 2.89 \times 10^{-4}$, $P = 0.99$). Interestingly, the total amount of food eaten during the hoarding session revealed that isolation- and grid-reared animals ate less than the grouped- and sawdust-reared rats, respectively. The overall ANOVA yielded significant main effects of rearing condition ($F_{1,28} = 8.6$,

$P < 0.01$) and caging condition ($F_{1,28} = 13.9$, $P < 0.001$), but no rearing × caging interaction ($F_{1,28} = 1.1$, $P = 0.3$).

Principal components analysis and partial least squares–discriminant analysis provide a first neurochemical map

A four component model was obtained which accounted for 90% of the variation in the data. The first two components are plotted in Fig. 5 (left panel). On the basis of both the PCA scores and corresponding loading plots, the brain regions were separated according to dopaminergic variables along the component PC[1] (horizontal, E-to-W or *x*-axis) and to serotonergic variables along component PC[2] (vertical, N-S or *y*-axis). The first component PC[1], dopaminergic, shows the separation between the NAC and CP to the right, from the amygdala, right and central, the INFRA, EC and hippocampus regions left and central, and the CING further to the left. The corresponding loading plot (Fig. 5, right panel) explains that the discrimination is due to greater levels of dopamine, DOPAC, HVA and DOPAC/HVA ratio to the right in the NAC and CP regions compared with the levels of the other regions to their left. Opposite to this, is that the CING region has a higher HVA/dopamine ratio, suggesting that in this region dopamine is preferentially catabolized by extraneuronal catechol-*O*-methyltransferase to HVA.

The second component PC[2], serotonergic, vertically separates the NAC and CP on the right and the hippocampus and CING on the left. The loading plot (Fig. 5, right panel) suggests that this separation is due to lower levels of 5-HT and higher 5-HIAA/5-HT ratios in the hippocampus and CP relative to the NAC, amygdala and CING.

The groupings of the tissues were further resolved by means of a partial least squares–discriminant analysis (PLS–DA). Figure 6 shows how the seven regions are separated according to both the dopaminergic and serotonergic variables. Since the PCA revealed such large regional variations and little evidence of other effects due to rearing condition or hemisphere at this region-to-region level, a separate PCA and PLS–DA was conducted for each of the seven brain regions.

Effect of isolation rearing and home caging conditions on neurochemical variables in the amygdala

In Fig. 7 it is shown that the isolates reared on grids can be clearly discriminated from the other groups along the dominant *x*-axis. The variables which contribute most significantly to this separation are HVA/dopamine, DOPAC/HVA together with dopamine and DOPAC. The PLS–DA provides evidence that the isolates reared on grids have an elevated HVA/dopamine ratio together with a lower DOPAC/HVA ratio and lower levels of both dopamine and DOPAC compared with the other groups, which have a similar neurochemical profile in the amygdala. There is also a suggestion of separation between the two hemispheres of isolates reared on grid floor along the *y*-axis due to increased average levels of HVA witnessed for these animals in their right hemisphere relative to their left counterpart. The multivariate analysis was supported by separate univariate analyses of each neurochemical variable. The 95% confidence intervals for the ratios of difference between isolates on grids and the control group for dopamine (0.147, 0.404) and DOPAC (0.284, 0.774) did not include one while the upper and lower limits were less than one, thus confirming that isolates on grids had lower levels of dopamine and DOPAC compared with the control group. The 95% confidence intervals for the ratios of difference between isolates on grids and the control group for the HVA/dopamine ratio (2.45, 7.06) did not include one while the lower limit was greater than one, thus verifying that isolates reared on grids had a significantly higher HVA/dopamine turnover in the amygdala compared with the control group. A lower DOPAC/HVA ratio was also apparent in isolates reared on grids ($P < 0.001$). Finally, isolates on grids had higher levels of HVA in the right hemisphere than in the left, which was confirmed by the 95% confidence intervals (1.896, 5.95) that did not include one while both upper and lower limits were greater than one.

Effect of isolation rearing and home caging conditions on neurochemical variables in the entorhinal cortex

For the EC, the PLS–DA and subsequent follow-up comparisons provided evidence that the DOPAC/HVA ratio of isolates reared on grids is predominantly lower than that of the control group ($P < 0.01$) (Fig. 8). In addition, the right hemisphere of isolates on grids showed significantly less 5-HIAA compared with the grouped on sawdust control group ($P < 0.01$). Isolates on grids also had higher levels of HVA and a higher HVA/dopamine ratio in the left hemisphere compared with the left hemisphere of controls. The 95% confidence intervals for the ratios of difference between isolates on grids and the control group for HVA (3.85, 14.12) and HVA/dopamine (1.538, 6.435) in the left hemisphere did not include one while both the lower and upper limit were greater than one. Finally, grouped on grids had higher levels of dopamine in both the left and right hemispheres compared with the control group, as revealed by the 95% confidence intervals for dopamine in the left (1.298, 3.143) and right (1.116, 2.621) hemispheres.

Effect of isolation rearing and home caging conditions on neurochemical variables in the hippocampus

The PCA and PLS–DA for the hippocampus provided evidence of decreased levels of HVA in the left hemisphere of isolates on sawdust compared with grouped-reared animals on sawdust ($P < 0.05$). The DOPAC/HVA ratio was significantly elevated in grouped on grids and isolates on sawdust compared with grouped on sawdust. Finally, the DOPAC/dopamine ratio was significantly elevated in the hippocampus of grouped on grids. A univariate analysis carried out separately on HVA, DOPAC/HVA and DOPAC/dopamine revealed statistically significant differences for HVA in the isolates on sawdust compared with the grouped on sawdust rats, for DOPAC/HVA between the grouped rats reared on a grid floor and the isolates on sawdust compared with the controls with a 5% level of significance, and for DOPAC/dopamine in the grouped rats reared on a grid floor vs the DOPAC/dopamine ratio in control rats with a 5% level of significance.

Effect of isolation rearing and home caging conditions on neurochemical variables in the anterior cingulate cortex

The PLS–DA for the CING showed that in this region there was separation between the isolates reared on a grid floor and the other groups due to an increase in HVA/dopamine as well

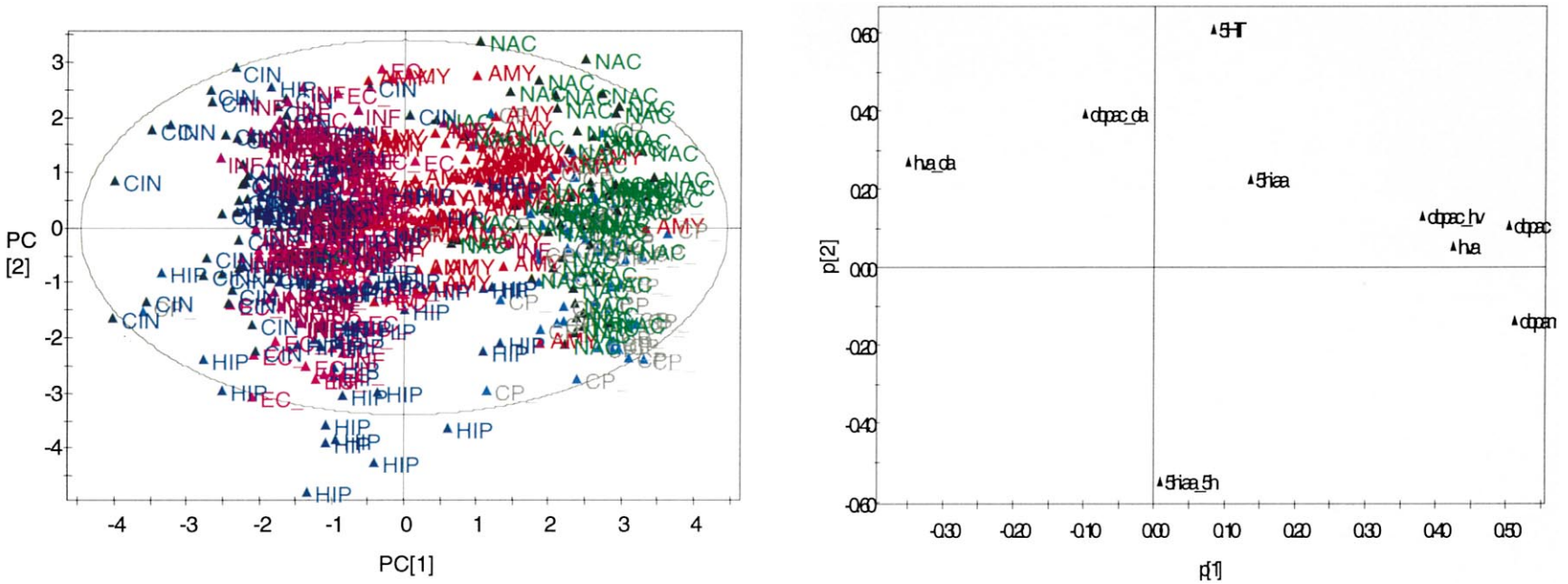

Fig. 5. PCA and dimensionally reduced expression of neurochemical data. All brain regions are plotted on to the first and second principal components (left panel). The first principal component (horizontal, E-to-W or *x*-axis) corresponds to dopaminergic variables (dopamine, DOPAC, HVA, DOPAC/dopamine, HVA/dopamine, DOPAC/HVA), the second (vertical, N–S or *y*-axis) represents serotonergic variables (5-HT, 5-HIAA, 5-HIAA/5-HT). The corresponding loading plot (right panel) represents the neurochemical basis of the separation between the NAC and CP to the right, from the amygdala, right and central, the INFRA, EC and hippocampus regions left and central, and the CING further to the left.

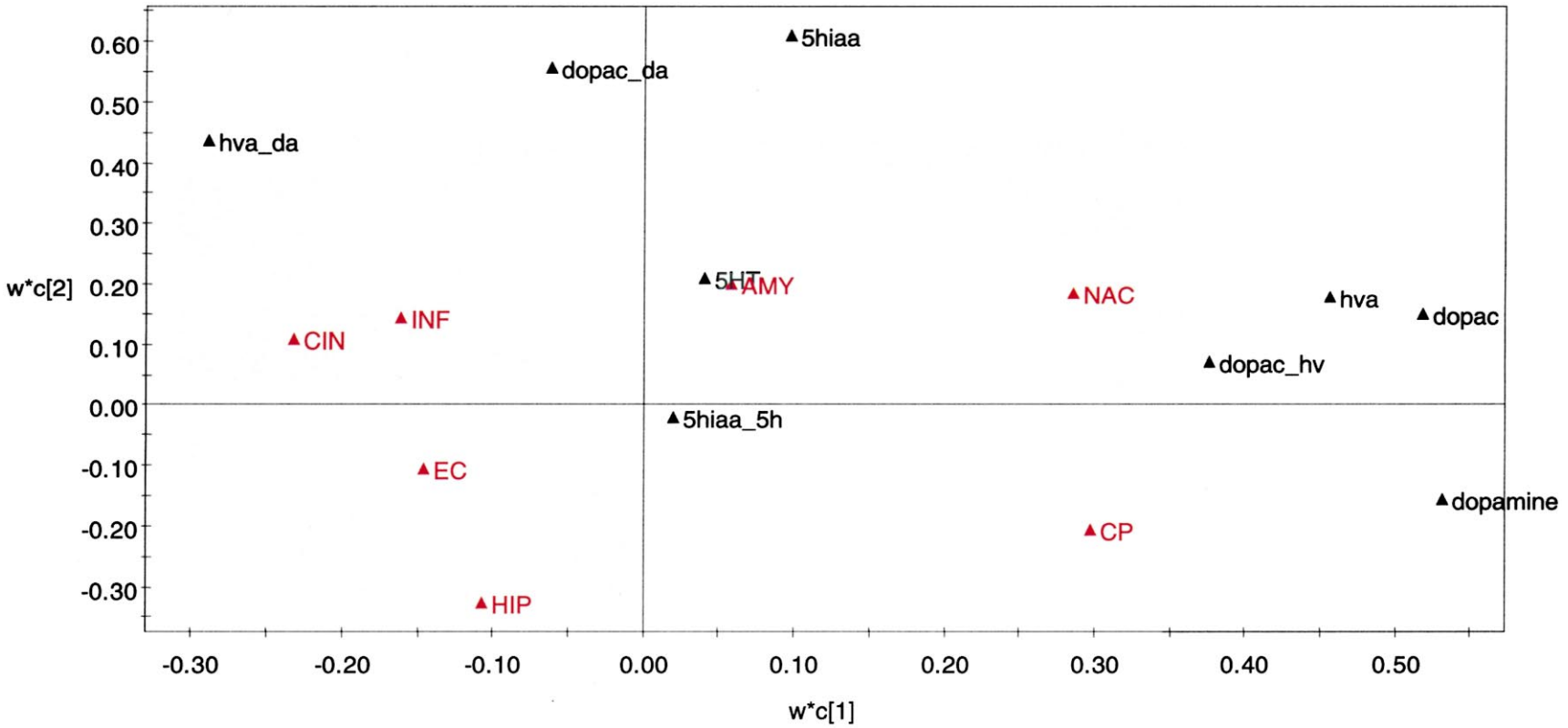

Fig. 6. PLS–DA and separation of the seven brain regions examined in the present study according to both the dopaminergic and serotonergic variables. The figure reveals that the discrimination between regions is due to greater levels of dopamine, DOPAC, HVA and DOPAC/HVA ratio to the right in the NAC and CP compared with the levels of the other regions to their left. Opposite to this, is that the CING and INFRA regions have a higher HVA/dopamine ratio, suggesting that in this region dopamine is preferentially catabolized by extraneuronal catechol-*O*-methyltransferase to HVA.

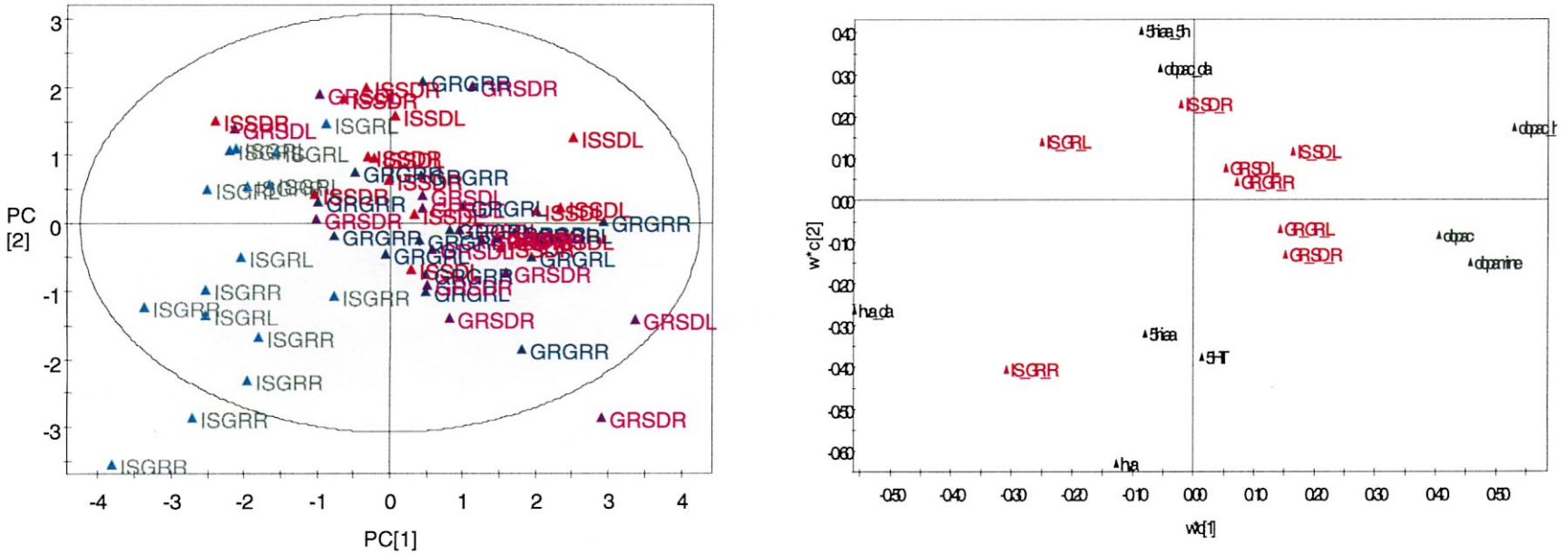

Fig. 7. PCA and corresponding loading plots in the amygdala. The figure represents the discrimination between the four experimental conditions and two hemispheres along the dopaminergic and serotonergic variables: grouped on sawdust right hemisphere [GR_SD_R], grouped on sawdust left hemisphere [GR_SD_L], grouped on grids right hemisphere [GR_GR_R], grouped on grids left hemisphere [GR_GR_L], isolates on sawdust right hemisphere [IS_SD_R], isolates on sawdust left hemisphere [IS_SD_L], isolates on grids right hemisphere [IS_GR_R], and isolates on grids left hemisphere [IS_GR_L].

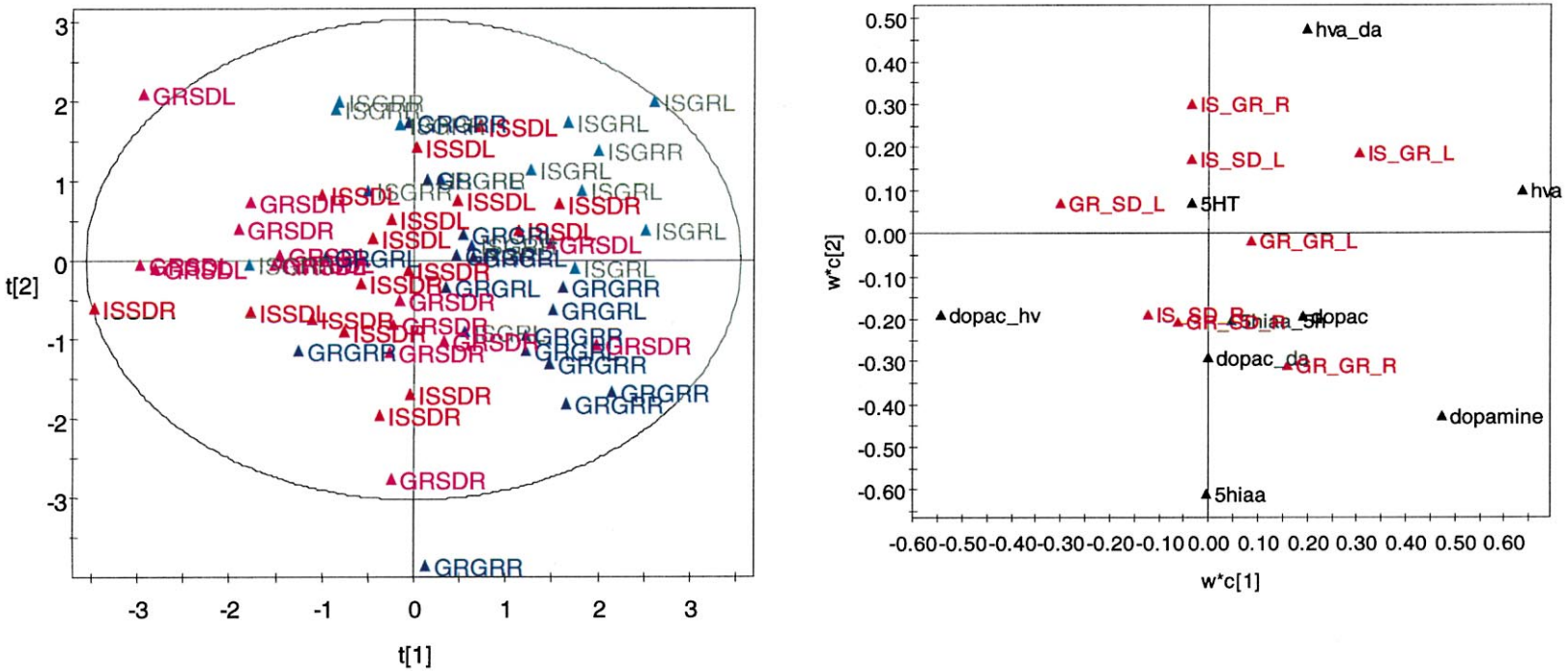

Fig. 8. PCA and corresponding loading plots in the entorhinal cortex. The figure represents the discrimination between the four experimental conditions and two hemispheres along the dopaminergic and serotonergic variables: grouped on sawdust right hemisphere [GR_SD_R], grouped on sawdust left hemisphere [GR_SD_L], grouped on grids right hemisphere [GR_GR_R], grouped on grids left hemisphere [GR_GR_L], isolates on sawdust right hemisphere [IS_SD_R], isolates on sawdust left hemisphere [IS_SD_L], isolates on grids right hemisphere [IS_GR_R], and isolates on grids left hemisphere [IS_GR_L].

as a decrease in DOPAC/HVA, which both were found to be statistically significant by the univariate analysis ($P < 0.01$). The isolates on grids also showed significantly different levels of HVA compared with grouped on sawdust ($P < 0.05$). Finally, a significant increase in the DOPAC/dopamine ratio was found in the CING of grouped on grids compared with control animals ($P < 0.01$).

Effect of isolation rearing and home caging conditions on neurochemical variables in the infralimbic cortex

From the PLS–DA for this region (Fig. 9), there was evidence of a separation of the isolates reared on grids (both hemispheres) due to HVA, separation of grouped rats reared on grids with contributions from DOPAC/dopamine and DOPAC/HVA, and also evidence of a separation of the isolates reared on sawdust (left hemisphere) with contributions from all three serotonergic variables. Univariate analyses showed that there was a significant increase in the DOPAC/dopamine ratio in grouped on grids ($P < 0.01$), whereas both isolates (sawdust and grids) showed a significantly decreased DOPAC/dopamine ratio compared with the grouped on sawdust control group ($P < 0.05$). Finally, isolates on sawdust had a significant decrease in 5-HIAA levels compared with grouped on sawdust ($P < 0.05$).

Effect of isolation rearing and home caging conditions on neurochemical variables in the nucleus accumbens

The PLS–DA provided evidence that in the NAC there was a clear difference in 5-HIAA between the isolates on sawdust (both hemispheres) and the grouped rats reared on sawdust as well as a separation due to 5-HT between the isolates reared on grid floors (both hemispheres) and the controls (Fig. 10).

The results of the univariate analyses show that there was a statistically significant decrease in 5-HIAA in isolates on sawdust compared with the control group. The 95% confidence intervals for the 5-HIAA data, which did not require a log transformation, yielded negative values (-240.7, -27.7) that did not contain zero.

Effect of isolation rearing and home caging conditions on neurochemical variables in the caudate–putamen

PCA provided evidence of a drop in DOPAC in this region for the isolates reared on sawdust. The univariate ANOVA for DOPAC and subsequent follow-up comparisons showed that there was a statistically significant reduction in DOPAC for the isolates on sawdust compared with grouped rats reared on sawdust ($P < 0.01$). Confidence intervals for the difference between the control group (grouped on sawdust) and other subgroups of rearing and caging conditions were calculated for the CP. Univariate analyses also revealed that the left hemisphere of isolates on grids contained lower levels of dopamine compared with grouped on sawdust ($P < 0.05$), whereas the left hemisphere of isolates on sawdust yielded a lower 5-HIAA/5-HT ratio ($P < 0.05$).

Isolation rearing and interhemispheric coupling coefficients for dopamine and serotonin

Interhemispheric coupling is assessed by determining correlation coefficients between neurotransmitters in the left and right hemispheres.[13,14] These correlations are listed in Table 1. It should be noted that the lack of group differences in mean neurochemical levels does not affect the interhemispheric coupling coefficient, since the correlations, which were calculated within the four experimental conditions, are statistically independent of the means of those groups.

Rearing rats in isolation produced a change in the interhemispheric coupling coefficient matrix for dopamine in the CING. Thus, isolates reared either in sawdust or in grid-floor cages showed a significant coupling coefficient in the dorsal part of the mPFC ($P < 0.0001$). In contrast, rearing rats in sawdust produced significant coupling coefficients in the hippocampus of both grouped and isolates ($P < 0.001$).

Effect of isolation rearing and home caging conditions on plasma levels of adrenocorticotrophic hormone and corticosterone

Despite the absence of an effect of either rearing condition ($F_{1,28} = 0.2$, $P = 0.7$) or caging condition ($F_{1,28} = 1.4$, $P = 0.2$) on ACTH levels, corticosterone levels were affected by caging conditions, with rats reared on grids having higher corticosterone than rats reared in sawdust cages ($F_{1,28} = 4.9$, $P < 0.03$). There was no main effect of rearing condition ($F_{1,28} = 2.6$, $P = 0.3$) and no rearing condition × caging condition interaction ($F_{1,28} = 1.1$, $P = 0.2$).

Correlations between plasma corticosterone levels and neurochemical events on left and right sides of the brain

Correlation coefficients between absolute levels of dopamine and 5-HT and plasma corticosterone were calculated for isolated and grouped rats reared either in sawdust-lined or in grid-floor cages. These correlations are listed in Tables 2 and 3. Among these, the highest significant positive correlation between corticosterone and dopamine was found in the left NAC of isolates reared on grids ($F_{1,7} = 36.5$, $P < 0.0009$; $Y = 7.207x - 1013.9$; $r = 0.93$; $r^2 = 0.86$). In grouped animals reared on sawdust, a significant correlation between dopamine and plasma corticosterone levels was observed in the right amygdala. Furthermore, grouped animals reared on grids showed a significant correlation between corticosterone levels and 5-HT in the right amygdala, whereas grouped animals reared in sawdust cages had significant correlations between corticosterone levels and 5-HT in the right CP. Finally, a significant correlation between corticosterone levels and 5-HT in the right amygdala was found in isolates reared on sawdust.

DISCUSSION

The results of the present study demonstrate that rearing rats in isolation (i) produces a syndrome of locomotor hyperactivity; (ii) increases the startle response and impairs PPI; (iii) increases basal dopamine turnover in the amygdala; (iv) decreases basal dopamine turnover in the INFRA; and (v) decreases basal turnover of 5-HT in the NAC. In the EC, dopamine neurotransmission appeared to be affected preferentially by caging conditions as a decreased basal turnover of dopamine was observed in grid-reared animals. Plasma corticosterone levels were also increased in grid-reared animals compared with rats reared in sawdust cages. Furthermore, social isolation resulted in an increased communication between the dopamine systems linking the two hemispheres at the level of the CING. Finally, isolates reared on grids showed

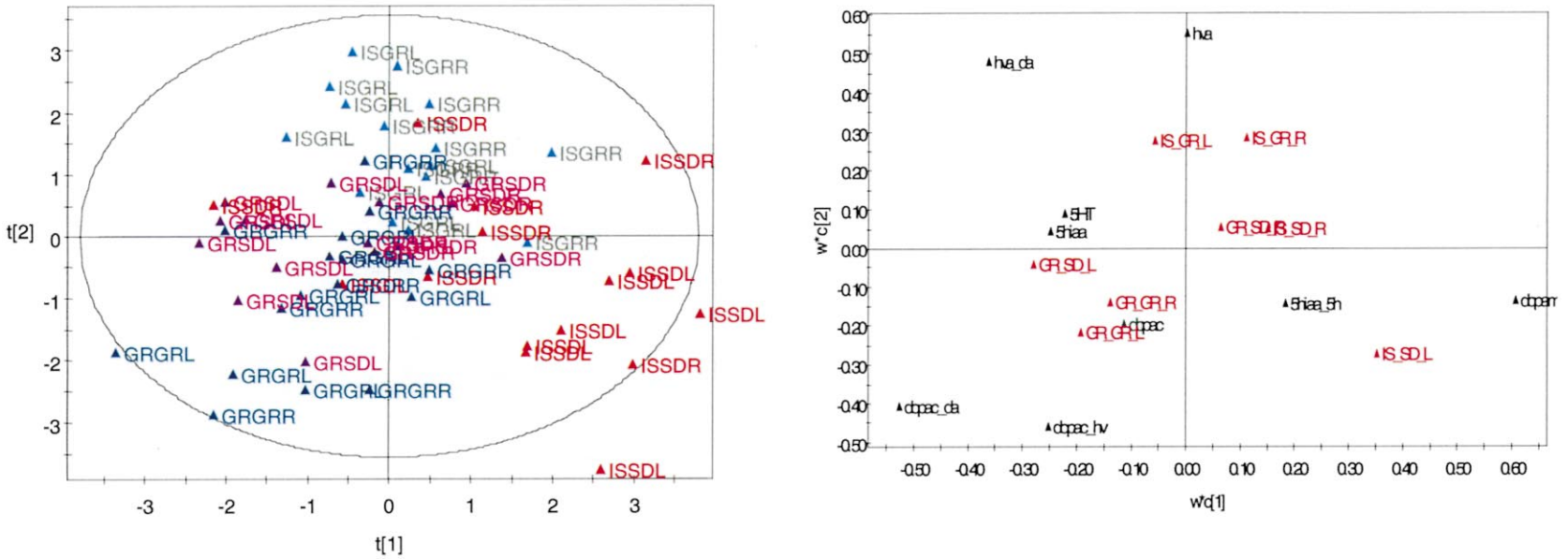

Fig. 9. PCA and corresponding loading plots in the infralimbic subregion of the medial prefrontal cortex. The figure represents the discrimination between the four experimental conditions and two hemispheres along the dopaminergic and serotonergic variables: grouped on sawdust right hemisphere [GR_SD_R], grouped on sawdust left hemisphere [GR_SD_L], grouped on grids right hemisphere [GR_GR_R], grouped on grids left hemisphere [GR_GR_L], isolates on sawdust right hemisphere [IS_SD_R], isolates on sawdust left hemisphere [IS_SD_L], isolates on grids right hemisphere [IS_GR_R], and isolates on grids left hemisphere [IS_GR_L].

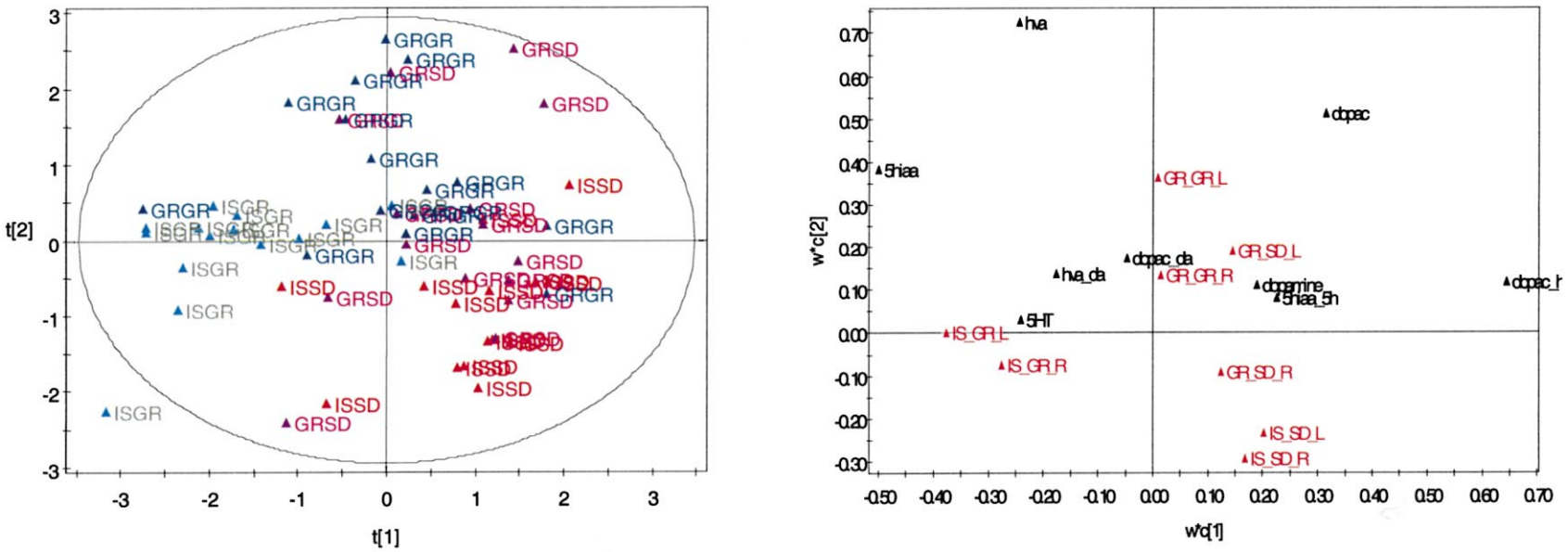

Fig. 10. PCA and corresponding loading plots in the nucleus accumbens. The figure represents the discrimination between the four experimental conditions and two hemispheres along the dopaminergic and serotonergic variables: grouped on sawdust right hemisphere [GR_SD_R], grouped on sawdust left hemisphere [GR_SD_L], grouped on grids right hemisphere [GR_GR_R], grouped on grids left hemisphere [GR_GR_L], isolates on sawdust right hemisphere [IS_SD_R], isolates on sawdust left hemisphere [IS_SD_L], isolates on grids right hemisphere [IS_GR_R], and isolates on grids left hemisphere [IS_GR_L].

Table 1. Interhemispheric coupling coefficients for dopamine (DA) and 5-hydroxytryptamine (5-HT) in the amygdala (AMY), entorhinal cortex (EC), dorsal hippocampus (HIPP), anterior cingulate cortex (CING), infralimbic cortex (INFRA), dorsolateral caudate–putamen (CP) and nucleus accumbens (NAC) of isolation-reared rats in each caging condition (sawdust vs grid-floor) compared with rats reared in social groups in similar caging conditions

	Grouped-reared				Isolation-reared			
	Grid		Sawdust		Grid		Sawdust	
	DA	5-HT	DA	5-HT	DA	5-HT	DA	5-HT
AMY	− 0.132	0.029	0.159	0.267	− 0.289	− 0.421	0.03	− 0.536
EC	0.409	− 0.631	− 0.431	− 0.33	− 0.167	0.394	− 0.174	− 0.476
HIPP	− 0.354	0.069	0.882*	0.672	− 0.313	− 0.499	0.785*	− 0.373
CING	-1.2×10^{-8}	− 0.707*	− 0.278	0.71	0.945*	0.271	1.0*	− 0.581
INFRA	-2.1×10^{-9}	− 0.452	− 0.526	0.197	-2.6×10^{-8}	− 0.461	0.038	− 0.316
CP	0.157	− 0.804*	0.099	0.057	0.824 *	− 0.276	0.586	0.751
NAC	0.582	− 0.163	0.038	− 0.646	− 0.631	− 0.43	0.639	− 0.182

*r significantly different from zero ($P < 0.05$).

Table 2. Correlations between both plasma corticosterone levels and dopamine in the amygdala (AMY), entorhinal cortex (EC), dorsal hippocampus (HIPP), anterior cingulate cortex (CING), infralimbic cortex (INFRA), dorsolateral caudate–putamen (CP) and nucleus accumbens (NAC) from both the left and right hemispheres of isolation-reared rats in each caging condition (sawdust vs grid-floor) compared with rats reared in social groups in similar caging conditions

	Grouped-reared				Isolation-reared			
	Grid		Sawdust		Grid		Sawdust	
	Left	Right	Left	Right	Left	Right	Left	Right
AMY	0.113	0.231	0.290	0.807*	0.206	0.038	0.354	0.469
EC	0.184	0.102	0.524	0.418	0.294	0.560	4.9×10^{-3}	0.192
HIPP	0.221	0.132	0.208	0.218	0.403	0.365	0.033	0.021
CING	0.292	0.214	0.109	0.679	0.063	0.027	0.211	0.211
INFRA	0.025	0.543	0.132	0.179	0.026	0.499	0.060	0.052
CP	0.124	0.505	0.313	0.596	0.012	0.158	0.066	0.202
NAC	0.723*	0.483	0.324	0.460	0.927*	0.601	0.110	0.263

*r significantly different from zero ($P < 0.05$).

Table 3. Correlations between both plasma corticosterone levels and 5-hydroxytryptamine (5-HT) in the amygdala (AMY), entorhinal cortex (EC), dorsal hippocampus (HIPP), anterior cingulate cortex (CING), infralimbic cortex (INFRA), dorsolateral caudate–putamen (CP) and nucleus accumbens (NAC) from both the left and right hemispheres of isolation-reared rats in each caging condition (sawdust vs grid-floor) compared with rats reared in social groups in similar caging conditions

	Grouped-reared				Isolation-reared			
	Grid		Sawdust		Grid		Sawdust	
	Left	Right	Left	Right	Left	Right	Left	Right
AMY	0.144	0.729*	0.217	0.101	0.452	0.176	0.289	0.799*
EC	0.549	0.059	0.782	0.418	0.168	0.110	0.696	0.509
HIPP	0.151	0.547	0.709	0.572	0.296	0.501	0.416	0.282
CING	0.062	0.201	0.152	0.560	0.038	0.212	0.569	0.327
INFRA	0.165	0.177	0.359	0.132	0.080	0.397	0.472	0.219
CP	0.084	0.334	0.213	0.905*	0.707	0.447	0.644	0.489
NAC	0.226	0.336	0.330	0.552	0.137	0.203	0.389	0.326

*r significantly different from zero ($P < 0.05$).

a significant positive correlation between plasma corticosterone levels and dopamine in the left NAC.

Effect of social isolation and home caging conditions on behavior

In agreement with previous reports in the literature,[17,26,85,86] the present study confirms that isolation-reared animals show spontaneous locomotor hyperactivity when exposed to the open field. Furthermore, the lack of interaction between the rearing and caging conditions reflects that the isolation-rearing induced effect on spontaneous locomotor activity occurs irrespective of the caging condition.

The present study demonstrates that there was no statistical evidence of a main effect of either caging or rearing conditions on the mean PPI response. However, a rearing × prepulse intensity interaction revealed a significant isolation-induced PPI deficit at the prepulse intensity of 84 dB[A]. In

contrast, caging conditions failed to affect either PPI or startle response. These results confirm the lack of isolation-induced disruption of mean PPI observed in Wistar rats[34] and the previously reported fragility of the phenomenon.[17,85] We have previously suggested[17,85] that several factors may influence the expression of a robust PPI deficit: the provenance of the animals (breeding facilities and husbandry conditions), the rat strain (Wistar vs Lister hooded, Sprague–Dawley or Fawn hooded rats), the number of prepulse intensities, and the number of PPI test sessions to which isolates must be exposed.

In the present study, there was a trend towards an additive effect of isolation and sawdust floors on increasing both the number and weight of pellets hoarded by rats on average. Disruptions of food hoarding behavior have been classically associated with bilateral neurotoxic or electrolytic lesions of the mPFC or basal forebrain.[12,45,47,48,76] Interestingly, bilateral electrolytic lesions of the dorsal, but not ventral, hippocampus appear to produce significant increases in food hoarding scores.[7] Thus, decreased dopamine levels together with increased dopamine turnover in the mPFC as a result of either small bilateral lesions of the ventral tegmental area or local damage to the mPFC lead to behavioral changes such as increased activity (both locomotion and exploratory behavior), impaired food hoarding and impaired spatial delayed alternation.[76] In the present study, decreased dopamine turnover in the prelimbic/infralimbic portion of the mPFC and decreased levels of HVA in the left dorsal hippocampus of isolates on sawdust may explain a tendency towards increased food hoarding in isolation-reared animals.

Effect of social isolation and home caging conditions on dopamine activity in the amygdaloid complex

In the present study, rearing rats in isolation and on grid floors produced a significant reduction in the steady-state levels of dopamine in the amygdala. An increased basal turnover of amygdala dopamine in isolates was associated with an increased and preferential catabolism of dopamine by extraneuronal catechol-*O*-methyltransferase to HVA, as reflected by both the HVA/dopamine and DOPAC/HVA ratios. Thus, rearing rats in isolation and on grid-floor cages was associated with a significant increase in basal turnover of dopamine in the amygdaloid complex. The amygdala is involved in the regulation and recognition of fear-motivated behavior.[1,49] Furthermore, the amygdaloid complex has a key role in the cardiovascular and neuroendocrine responses to stress.[3,4,21,25,65,70,83] Activation of the mesoamygdaloid dopamine system has been reported to occur during both the acquisition and expression of conditioned fear.[11,33] In addition, the magnitude of the dopamine response to stress induced by mild handling is significantly greater in the amygdala than in the NAC and mPFC.[38] Thus, our data suggest that increased dopamine turnover in the amygdala of rats reared on grids and in isolation may be an index of chronic mild stress.

A link between the social isolation syndrome, attentional deficits, locomotor hyperactivity and dopamine dynamics in the medial prefrontal cortex

Our findings indicate that basal turnover of dopamine was decreased in the INFRA of isolates reared in both home caging conditions. Specifically, increased steady-state levels of dopamine were associated with a decreased catabolism of dopamine by intraneuronal monoamine oxidase to DOPAC as reflected by the DOPAC/dopamine and DOPAC/HVA ratios. Altogether, the results of the present study suggest that the basal turnover of dopamine is decreased in the mPFC of rats reared in isolation. These alterations were specific to cortical dopamine, as there were no significant changes in the NAC or CP. Selective changes in cortical dopamine function following social isolation have been reported in previous studies.[6,36,43] Although there are some discrepancies between these experiments, the general consensus is that social isolation produces increased dopamine levels, but decreased DOPAC/dopamine ratios in the PFC. We have demonstrated with others[17,26,30,42,69,85,86] that rats reared in social isolation are spontaneously hyperactive and show deficits in PPI of the acoustic startle response, which represents a deficit in attentional performance. Interestingly, decreased DOPAC/dopamine ratios have been reported in the mPFC of rats with low choice accuracy in the five-choice serial reaction time task as a model of attention deficit hyperactivity disorder.[63] Furthermore, animals with higher locomotor responses to novelty, which are usually referred to as high responders, tend to acquire amphetamine self-administration and show a reduced DOPAC/dopamine ratio in the mPFC.[61] Thus, there seems to be a link between social isolation, attention deficit, spontaneous locomotor hyperactivity and reduced dopamine turnover in the mPFC. Our results further demonstrate that this altered dopamine function in isolated rats can be observed preferentially in the ventral cytoarchitectonic subdivision of the mPFC. Given that a critical concentration of dopamine is required for normal function of the mPFC,[9,55,71] it is reasonable to proceed on the working hypothesis that a change in dopamine activity in the mPFC of isolated rats may produce altered selectivity or sharpening of stimuli to apical dendrites and basal dendrites/soma.

Chronic mild stress, plasma corticosterone levels and dopamine activity in the medial prefrontal cortex, entorhinal cortex and nucleus accumbens

Our results demonstrate that the DOPAC/dopamine ratio was increased in the mPFC (both CING and INFRA) of animals reared in social groups on grids. Profound alterations in dopamine systems have been reported after the acute or repeated exposure to stressors. For instance, footshock stress has been shown to produce a significant elevation of dopamine turnover in the mesocortical system.[37,39,64,75] Furthermore, prenatal stress has been shown to increase dopamine turnover in the right PFC.[23] Thus, one may hypothesize that rearing rats on grids represents a form of chronic mild stress that would lead via increased dopamine turnover in the mesocortical system to increased activity of the mPFC. Interestingly, a significant positive correlation between dopamine in the left NAC and plasma corticosterone levels was found in the present study in both grouped and isolated rats reared on grids. It has been shown that the peripheral administration of corticosterone at a dose that corresponds to stress-induced plasma corticosterone levels produces enhanced dopamine levels in the NAC.[62] These results, together with the findings of the present study, suggest that the higher the plasma corticosterone levels induced by chronic mild stress such as rearing rats in grid-floor cages, the higher the dopamine concentrations in the

NAC. In addition to the significant relationship between plasma corticosterone levels and dopamine in the left NAC, increased levels of dopamine in the EC of grouped on grids were associated with a decreased catabolism of dopamine by intraneuronal monoamine oxidase to DOPAC, as reflected by a decreased DOPAC/HVA ratio. A preferentially left lateralized functional interdependence between the EC and NAC has been demonstrated.[51] Specifically, unilateral 6-hydroxydopamine lesions of the right EC produce an increased basal turnover of dopamine in the left NAC.[50] With regard to the interesting effect of caging on corticosterone and not on ACTH, this would suggest adrenal hyper-sensitivity to ACTH, and therefore chronic stress, in rats maintained on grids. Elevated circulating glucocorticoid in association with normal ACTH levels is a characteristic of human depression, and has been interpreted as the result of long-term overstimulation of the adrenal cortex by ACTH, whereas levels of the latter are eventually reduced by down-regulation of CRF receptors on the corticotrophs.[36,56] Thus, our findings suggest that rearing rats in grid-floor cages represents a chronic mild stress that would be associated with increased basal corticosterone levels, decreased basal turnover of entorhinal dopamine, and increased dopamine activity in the left NAC.

The social isolation syndrome and serotonin dynamics in the nucleus accumbens

In the present study, isolates on grids showed a significant decrease in the basal turnover of 5-HT in the NAC. These results are in line with previous studies showing that both *post-mortem* 5-HIAA/5-HT ratios and extracellular levels of 5-HT are reduced in the NAC of isolated rats.[41,43] The spontaneous locomotor hyperactivity observed in isolated rats may be explained, at least in part, by the reduction in serotonergic activity in the NAC. It has been shown that 5-HT activity has an inhibitory effect on locomotion,[32,80] whereas 5,7-dihydroxytryptamine lesions of the NAC increase both spontaneous and amphetamine-induced locomotor activity.[10,52] Furthermore, increasing 5-HT activity seems to decrease the isolation-induced hyperactivity.[18,57] Finally, recent immunoelectron microscopy studies suggest that 5-HT-immunoreactive axon terminals in the NAC form symmetric synaptic junctions with postsynaptic neurons and that 5-HT- and GABA-immunoreactive axon terminals are in direct apposition to one another.[82] Dopaminergic terminals in the NAC make symmetric contacts with GABAergic medium spiny neurons,[73] whereas prefrontal cortical afferents, presumably glutamatergic in nature, make asymmetric synapses on the same medium spiny neurons.[73] Both 5-HT and dopamine axons also converge on the same neurons in the medial[8,81] and caudal one-third of the NAC.[59] Thus, one may assume that rearing rats in isolation modifies the balance between dopamine and 5-HT neurotransmission systems in the NAC. Whether isolation rearing produces a change in the activity of GABAergic NAC output neurons to the ventral pallidum requires further investigations.

Effect of social isolation and home caging conditions on interhemispheric coupling coefficients

Our results show that social isolation resulted in an increased communication between the dopamine systems linking the two hemispheres at the level of the CING. Furthermore, animals reared on sawdust showed positive interhemispheric dopaminergic coupling between the hippocampi. The presence of significant positive correlations between measures of the right and left hemispheres is evidence for a negative feedback loop linking the two hemispheres.[13] It has been hypothesized that such correlations are indicative of a different brain organization, compared with brains that lack these correlations.[16] The left hemisphere is involved in communicative functions, there is a preferential involvement of the right hemisphere in affective and spatial information,[14] and both hemispheres interact through activation–inhibition mechanisms when emotional processes are involved.[15] Thus, the lack of significant correlations implies that the two hemispheres are operating independently due to an alteration in interhemispheric communication, whereas the hemispheres linked by a significant correlation are coupled via a hypothetical negative feedback loop.[16] In the present study, the group differences in the degree of neurochemical relationship between the two hemispheres indicate that isolation-reared animals have more interhemispheric coupling for dopamine, that is more dopaminergic communication, between both left and right CING. Whether these interhemispheric differences are causally related to the early social isolation syndrome remains to be determined by future research.

CONCLUSIONS

The present study has demonstrated that social isolation is characterized by a syndrome of generalized locomotor hyperactivity and increased startle response together with alterations in both PPI and food hoarding behavior. This syndrome also led to reduced dopamine turnover in the mPFC, which could be a mediating mechanism between social isolation and two of its behavioral effects, namely attention deficit and spontaneous locomotor hyperactivity. Furthermore, our data demonstrate that rearing rats in grid-floor cages represents a form of chronic mild stress that would be associated with increased corticosterone levels, increased basal dopamine turnover in the amygdala, decreased basal turnover of entorhinal dopamine and increased dopamine activity in the left NAC. A significant decrease in the basal turnover of 5-HT in the NAC of isolates on grids may also be linked to the isolation-induced locomotor hyperactivity. Finally, the present study points to the advantage of coupling univariate with multivariate data analysis techniques, such as PCA and PLS–DA to determine key variables and to simplify both the analysis and visualization of multidimensional data sets.

Acknowledgements—This study was performed in partial fulfilment of the requirements for the Ph.D. degree of Isabelle Weiss from the ETH Zürich and was supported by the Swiss Federal Institute of Technology (ETH) Zürich and the Swiss National Science Foundation (Grant no. 3100-051657). Isabelle Weiss was supported by a grant from Hoffmann-LaRoche (Basel, Switzerland). The authors gratefully acknowledge the insightful comments and criticisms of Dr Rebeca Heidbreder, the expert technical assistance of Mr Peter Schmid, and Dr Isabelle Allmann's team for excellent care of the animals used in the present study.

REFERENCES

1. Adolphs R., Tranel D., Damasio H. and Damasio A. (1994) Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature* **372,** 669–672.
2. Anisman H. and Zacharko R. M. (1982) Depression: the predisposing influence of stress. *Behav. Brain Sci.* **5,** 89–137.
3. Beaulieu S., DiPaolo T. and Barden N. (1986) Control of ACTH secretion by the central nucleus of the amygdala: implications of the serotoninergic system and its relevance to the glucocorticoid delayed negative feedback mechanism. *Neuroendocrinology* **44,** 247–254.
4. Beaulieu S., DiPaolo T., Cote J. and Barden N. (1987) Participation of the central amygdaloid nucleus in the response of adrenocorticotropin secretion to immobilization stress: opposing roles of the noradrenergic and dopaminergic systems. *Neuroendocrinology* **45,** 37–46.
5. Beaumont G. and Dimond S. (1973) Brain disconnection and schizophrenia. *Br. J. Psychiatry* **123,** 661–662.
6. Blanc G., Herve D., Simon H., Lisoprawski A., Glowinski J. and Tassin J. P. (1980) Response to stress of mesocortical–frontal dopaminergic neurons in rats after social isolation. *Nature* **284,** 265–267.
7. Borker A. S. and Mascarenhas J. F. (1990) Variations in hoarding behavior following regional hippocampal lesions in rats. *Indian J. exp. Biol.* **28,** 91–92.
8. Bouyer J. J., Joh T. H. and Pickel V. M. (1984) Ultrastructural localization of tyrosine hydroxylase in rat nucleus accumbens. *J. comp. Neurol.* **227,** 92–103.
9. Brozoski T. J., Brown R. M., Rosvold H. E. and Goldman P. S. (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science* **205,** 929–932.
10. Carter C. J. and Pycock C. J. (1979) The effects of 5,7-dihydroxytryptamine lesions of extrapyramidal and mesolimbic sites on spontaneous and amphetamine-induced stereotypy. *Naunyn-Schmiedenberg's Arch. Pharmac.* **308,** 51–54.
11. Coco M. L., Kuhn C. M., Ely T. D. and Kilts C. D. (1992) Selective activation of mesoamygdaloid dopamine neurons by conditioned stress: attenuation by diazepam. *Brain Res.* **590,** 39–47.
12. de Brabander J. M., de Bruin J. P. and van Eden C. B. (1991) Comparison of the effects of neonatal and adult medial prefrontal cortex leions on food hoarding and spatial delayed alternation. *Behav. Brain Res.* **42,** 67–75.
13. Denenberg V. H. (1980) General systems theory, brain organization, and early experience. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol. 7)* **238,** R3–R13.
14. Denenberg V. H. (1981) Hemispheric laterality in animals and the effects of early experience. *Behav. Brain Sci.* **4,** 1–49.
15. Denenberg V. H. (1983) Lateralization of function in rats. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol. 14)* **245,** R505–R509.
16. Denenberg V. H. and Rosen G. D. (1983) Interhemispheric coupling coefficients: sex differences in brain neurochemistry. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol. 14)* **245,** R151–R153.
17. Domeney A. and Feldon J. (1998) The disruption of prepulse inhibition by social isolation in the Wistar rat: how robust is the effect? *Pharmac. Biochem. Behav.* **59,** 883–890.
18. Einon D. F. and Morgan M. J. (1977) A critical period for social isolation in the rat. *Devl Psychobiol.* **10,** 123–132.
19. Einon D. F., Morgan M. J. and Sahakian B. J. (1975) The development of intersession habituation and emergence in socially reared and isolated rats. *Devl Psychobiol.* **8,** 553–559.
20. Einon D. F., Stewart J., Atkinson S. and Morgan M. J. (1976) The effects of isolation on barbiturate induced anaesthesia in the rat. *Psychopharmacology* **50,** 85–88.
21. Feldman S. and Conforti N. (1981) Amygdalectomy inhibits adrenocrotical responses to somatosensory and olfactory stimulation. *Neuroendocrinology* **32,** 330–334.
22. Fone K. C. F., Shalders K., Fox Z. D., Arthur R. and Marsden C. A. (1996) Increased 5-HT_{2C} receptor responsiveness occurs on rearing rats in social isolation. *Psychopharmacology* **123,** 346–352.
23. Fride E. and Weinstock M. (1988) Prenatal stress increases anxiety related behavior and alters cerebral lateralization of dopamine activity. *Life Sci.* **42,** 1059–1065.
24. Fulford A. J. and Marsden C. A. (1998) Conditioned release of 5-hydroxytryptamine *in vivo* in the nucleus accumbens following isolation-rearing in the rat. *Neuroscience* **83,** 481–487.
25. Galeno T. M., VanHoesen G. W. and Brody M. J. (1984) Central amygdaloid nucleus lesion attenuates exaggerated hemodynamic responses to noise stress in the spontaneously hypertensive rat. *Brain Res.* **291,** 249–259.
26. Gentsch C., Lichtsteiner M. and Feer H. (1981) Locomotor activity, defecation score, and corticosterone levels during an openfield exposure: a comparison among individually and group-housed rats, and genetically selected rat lines. *Physiol. Behav.* **27,** 183–186.
27. Gentsch C., Lichtsteiner M. and Feer H. (1982) Different reaction patterns in individually and socially reared rats during exposures to novel environment. *Behav. Brain Res.* **4,** 45–54.
28. Gentsch C., Lichtsteiner M. and Feer H. (1982) Behavioural comparisons between individually and group-housed male rats: effects of novel environments and diurnal rhythm. *Behav. Brain Res.* **6,** 93–100.
29. Gentsch C., Lichtsteiner M. and Feer H. (1983) Individually housed rats exceed group-housed animals in rotational movements when exposed to a novel environment. *Experientia* **39,** 1412–1414.
30. Geyer M. A., Wilkinson L. S., Humby T. and Robbins T. W. (1993) Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. *Biol. Psychiat.* **34,** 361–372.
31. Grace A. A. (2000) Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Res. Rev.* **31,** 330–341.
32. Green A. R., Gillin J. C. and Wyatt R. J. (1976) The inhibitory effects of intraventricular administration of serotonin on spontaneous motor activity of rats. *Psychopharmacology* **51,** 81–84.
33. Guarraci F. A., Frohard R. J. and Kapp B. S. (1999) Amygdaloid D1 dopamine receptor involvement in Pavlovian fear conditioning. *Brain Res.* **827,** 28–40.
34. Hall F. S., Huang S. and Fong G. (1997) Effects of isolation-rearing on acoustic startle and pre-pulse inhibition in Wistar and Fawn hooded rats. *Ann. N.Y. Acad. Sci.* **821,** 542–544.
35. Hatta T., Yamamoto M. and Kawabata Y. (1984) Functional hemispheric differences in schizophrenia: interhemispheric transfer deficit or selective hemispheric dysfunction. *Biol. Psychiat.* **19,** 1027–1036.
36. Holsboer F. (1989) Psychiatric implications of altered limbic–hypothalamic–pituitary–adrenocortical activity. *Eur. Arch. psychiat. neurol. Sci.* **238,** 302–322.
37. Holson R. R., Ali S. F. and Scallet A. C. (1988) The effect of isolation rearing and stress on monoamines in forebrain nigrostriatal, mesolimbic, and mesocortical dopamine systems. *Ann. N.Y. Acad. Sci.* **537,** 512–514.
38. Inglis F. M. and Moghaddam B. (1999) Dopaminergic innervation of the amygdala is highly responsive to stress. *J. Neurochem.* **72,** 1088–1094.
39. Inoue T., Tsuchiya K. and Koyama T. (1994) Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. *Pharmac. Biochem. Behav.* **49,** 911–920.
40. Jackson J. E. (1991) *A User's Guide to Principal Components*. John Wiley, New York.
41. Jones D. L., Mogenson G. J. and Wu M. (1981) Injection of dopaminergic, cholinergic, and GABAergic drugs into the nucleus accumbens: effects on locomotor activity in the rat. *Neuropharmacology* **20,** 29–37.
42. Jones G. H., Marsden C. A. and Robbins T. W. (1990) Increased sensitivity to amphetamine and reward-related stimuli following social isolation of rats: possible disruption of dopamine-dependent mechanisms of the nucleus accumbens. *Psychopharmacology* **102,** 364–372.

43. Jones G. H., Hernandez T. D., Kendall D. A., Marsden C. A. and Robbins T. W. (1992) Dopaminergic and serotonergic function following isolation rearing in rats: study of behavioural responses and postmortem and *in vivo* neurochemistry. *Pharmac. Biochem. Behav.* **43,** 17–35.
44. Juraska J. M., Greenough W. T. and Conlee J. W. (1983) Differential rearing affects responsiveness of rats to depressant and convulsant drugs. *Physiol. Behav.* **31,** 711–715.
45. Kalsbeek A., de Bruin J. P. C., Feenstra M. G. P., Mattijssen M. A. H. and Uylings H. B. M. (1988) Neonatal thermal lesions of the mesolimbocortical dopaminergic projection decrease food-hoarding behavior. *Brain Res.* **475,** 80–90.
46. Katz D. M. and Steinberg H. (1972) Factors which might modify morphine dependence in rats. In *Biochemical and Pharmacological Aspects of Dependence and Reports on Marijuana Research* (eds Van Praag H. M., De Ewen F. and Bohn N. V.). De Erven, Bonn.
47. Kolb B. (1974) Prefrontal lesions after eating and hoarding behavior in rats. *Physiol. Behav.* **12,** 507–511.
48. Lacroix L., Broersen L. M., Weiner I. and Feldon J. (1998) The effect of excitotoxic lesion of the medial prefrontal cortex on latent inhibition, prepulse inhibition, food hoarding, elevated plus maze, active avoidance and locomotor activity in the rat. *Neuroscience* **84,** 431–442.
49. LeDoux J. E. (1995) Emotion: clues from the brain. *A. Rev. Psychol.* **46,** 209–235.
50. Louilot A. and Choulli M. K. (1997) Asymmetrical increases in dopamine turnover in the nucleus accumbens and lack of changes in locomotor responses following unilateral dopaminergic depletions in the entorhinal cortex. *Brain Res.* **778,** 150–157.
51. Louilot A. and Le Moal M. (1994) Lateralized interdependence between limbicotemporal and ventrostriatal dopaminergic transmission. *Neuroscience* **59,** 495–500.
52. Lyness W. H. and Moore K. E. (1981) Destruction of 5-hydroxytryptaminergic neurons and the dynamics of dopamine in nucleus accumbens septi and other forebrain regions of the rat. *Neuropharmacology* **20,** 327–334.
53. Mogenson G. J., Jones D. L. and Yin C. Y. (1980) From motivation to action: functional interface between the limbic system and the motor system. *Prog. Neurobiol.* **14,** 69–97.
54. Morgan M. J., Einon D. and Morris R. G. M. (1977) Inhibition and isolation rearing in the rat: extinction and satiation. *Physiol. Behav.* **18,** 1–5.
55. Murphy B. L., Arnsten A. F. T., Goldman-Rakic P. S. and Roth R. H. (1996) Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. *Proc. natn. Acad. Sci. USA* **93,** 1325–1329.
56. Nemeroff C. B. (1992) New vistas in neuropeptide research in neuropsychiatry: focus on corticotropin-releasing factor. *Neuropsychopharmacology* **6,** 69–75.
57. Ogren S. O., Holm A. C., Renyi A. L. and Ross S. B. (1980) Antiaggressive effect of zimelidine in isolated mice. *Acta pharmac. toxicol.* **47,** 71–74.
58. Palkovits M. and Brownstein M. J. (1988) *Maps and Guide to Microdissection of the Rat Brain*. Elsevier, New York.
59. Phelix C. F. and Broderick P. A. (1995) Light microscopic immunocytochemical evidence of converging serotonin and dopamine terminals in ventrolateral nucleus accumbens. *Brain Res. Bull.* **37,** 37–40.
60. Phillips G. D., Howes S. R., Whitelaw R. B., Wilkinson L. S., Robbins T. W. and Everitt B. J. (1994) Isolation rearing enhances the locomotor response to cocaine and a novel environment, but impairs the intravenous self-administration of cocaine. *Psychopharmacology* **115,** 407–418.
61. Piazza P. V., Deminiere J. M., Le Moal M. and Simon H. (1989) Factors that predict individual vulnerability to amphetamine self-administration. *Science* **245,** 1511–1513.
62. Piazza P. V., Rouge-Pont F., Deroche V., Maccari S., Simon H. and Le Moal M. (1996) Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. *Proc. natn. Acad. Sci. USA* **93,** 8716–8720.
63. Puumala T. and Sirviö J. (1998) Changes in activities of dopamine and serotonin in the frontal cortex underlie poor choice accuracy and impulsivity of rats in an attention task. *Neuroscience* **83,** 489–499.
64. Reinhard J. F., Jr, Bannon M. J. and Roth R. H. (1982) Acceleration by stress of dopamine synthesis and metabolism in prefrontal cortex: antagonism by diazepam. *Naunyn-Schmiedeburg's Arch. Pharmac.* **318,** 374–377.
65. Roozendaal B., Koolhaas J. M. and Bohus B. (1992) Central amygdaloid involvement in neuroendocrine correlates of conditioned stress responses. *J. Neuroendocr.* **4,** 483–489.
66. Sahakian B. J. and Robbins T. W. (1977) Isolation-rearing enhances tail pinch-induced oral behaviours in rats. *Physiol. Behav.* **18,** 53–58.
67. Sahakian B. J., Robbins T. W., Morgan M. J. and Iversen S. D. (1975) The effects of psychomotor stimulants on stereotypy and locomotor activity in socially deprived and control rats. *Brain Res. Bull.* **84,** 195–205.
68. Sahakian B. J., Robbins T. W. and Iversen S. D. (1977) The effects of isolation on exploration in the rat. *Anim. Learn Behav.* **5,** 193–198.
69. Sahakian B. J., Burdess C., Luckhurst H. and Trayhurn P. (1982) Hyperactivity and obesity: the interaction of social isolation and cafetaria feeding. *Physiol. Behav.* **28,** 117–124.
70. Sanders B. J., Wirtz-Nole C., DeFord S. M. and Erling B. F. (1994) Central amygdaloid lesions attenuate cardiovascular responses to acute stress in rats with borderline hypertension. *Physiol. Behav.* **56,** 709–713.
71. Sawaguchi T. and Goldman-Rakic P. S. (1994) The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J. Neurophysiol.* **71,** 515–528.
72. Schenk S., Lacelle G., Gorman K. and Amit Z. (1987) Cocaine self-administration in rats influenced by environmental conditions: implications for etiology of drug abuse. *Neurosci. Lett.* **81,** 227–231.
73. Sesack S. R. and Pickel V. M. (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J. comp. Neurol.* **320,** 145–160.
74. Smith J. K., Neill J. C. and Costall B. (1997) Post-weaning housing conditions influence the behavioural effects of cocaine and d-amphetamine. *Psychopharmacology* **131,** 23–33.
75. Speciale S. G., Miller J. D., McMillen B. A. and German D. C. (1986) Activation of specific central dopamine pathways: locomotion and footshock. *Brain Res. Bull.* **16,** 33–38.
76. Stam C. J., de Bruin J. P. C., van Haelst A. M., van der Gugten J. and Kalsbeek A. (1989) Influence of the mesocortical dopamineric system on activity, food hoarding, social-agonistic behavior, and spatial delayed alternation in male rats. *Behav. Neurosci.* **103,** 24–35.
77. Swanson L. W. (1992) *Brain Maps: Structure of the Rat Brain*. Elsevier, New York.
78. Tamminga C. A., Thaker G. K., Buchanan R., Kirkpatrick B., Alphs L. D., Chase T. N. and Carpenter W. T. (1992) Limbic system abnormalities identified in schizophrenia using positron emission tomography with fluorodeoxyglucose and neocortical alterations with deficit syndrome. *Arch. gen. Psychiatry* **49,** 522–530.
79. Thoa N. B., Tizabi Y. and Jacobowitz D. M. (1977) The effect of isolation on the catecholamine concentration and turnover in discrete areas of the rat brain. *Brain Res.* **131,** 259–269.
80. Thornton E. W. and Goudie A. J. (1978) Evidence for the role of serotonin in the inhibition of specific motor responses. *Psychopharmacology* **60,** 73–79.
81. Van Bockstaele E. J. and Pickel V. M. (1993) Ultrastructure of serotonin-immunoreactive terminals in the core and shell of the rat nucleus accumbens: Cellular substrates for interactions with catecholamine afferents. *J. comp. Neurol.* **334,** 603–617.
82. Van Bockstaele E. J., Chan J. and Pickel V. M. (1996) Pre- and postsynaptic sites for serotonin modulation of GABA-containing neurons in the shell region of the rat nucleus accumbens. *J. comp. Neurol.* **371,** 116–128.
83. Van de Kar L. D., Piechowski R. A., Rittenhouse P. A. and Gray T. S. (1991) Amygdaloid lesions: differential effect on conditioned stress and immobilization-induced increases in corticosterone and renin secretion. *Neuroendocrinology* **54,** 89–95.
84. Varty G. B. and Higgins G. A. (1994) Differences between three rat strains in sensitivity to prepulse inhibition of an acoustic startle response: influence of apomorphine and phencyclidine pretreatment. *J. Psychopharmac.* **8,** 148–156.

85. Weiss I. C., Feldon J. and Domeney A. M. (1999) Isolation rearing-induced disruption of prepulse inhibition: further evidence for fragility of the response. *Behav. Pharmac.* **10,** 139–149.
86. Wilkinson L. S., Killcross S. S., Humby T., Hall F. S., Geyer M. A. and Robbins T. W. (1994) Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. *Neuropsychopharmacology* **10,** 61–72.
87. Wongwitdecha N. and Marsden C. A. (1995) Isolation rearing prevents the reinforcing properties of amphetamine in a conditioned place preference paradigm. *Eur. J. Pharmac.* **279,** 99–103.
88. Wright I. K., Ismail H., Upton N. and Marsden C. A. (1991) Effect of isolation-rearing on 5-HT agonist-induced responses in the rat. *Psychopharmacology* **105,** 259–263.
89. Yamamoto M. and Hatta T. (1984) Cerebral functional asymmetry and MBD children: a synergistic deficit of hemispheres. *Int. J. Neurosci.* **24,** 143–148.
90. Yang C. R. and Mogenson G. J. (1984) Electrophysiological responses of neurones in the nucleus accumbens to hippocampal stimulation and the attenuation of the excitatory responses by mesolimbic dopaminergic system. *Brain Res.* **324,** 69–84.
91. Yin C. Y. and Mogenson G. J. (1982) Responses of nucleus accumbens neurones to amygdala stimulation and its modification by dopamine. *Brain Res.* **239,** 401–415.

(*Accepted* 28 *June* 2000)