Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 83 (2006) 28 – 34
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The effects of social isolation on neuropeptide Y levels, exploratory and anxiety-related behaviors in rats

Annika Thorsell [a], Craig J. Slawecki [a], Aram El Khoury [b], Aleksander A. Mathe [b], Cindy L. Ehlers [a,*]

[a] The Scripps Research Institute, Department of Neuropharmacology (CVN-14), 10550 N. Torrey Pines Rd, La Jolla, CA 92037, USA
[b] Karolinska Institutet, Department of Pharmacology, Psychiatry, Huddinge University Hospital, SE-141 86 Stockholm, Sweden

Received 22 July 2005; received in revised form 30 November 2005; accepted 6 December 2005
Available online 3 February 2006

Abstract

NPY is one of the most abundantly expressed peptides within the CNS, and has been previously demonstrated to be altered in several animal models of depression, as well as to be differentially regulated by acute and repeated stress. The effect of social deprivation, through isolation housing, on brain NPY concentrations in adult rats has not been previously investigated. The effects of 12 weeks of social isolation, in adult rats, on anxiety-related behaviors and central concentrations of NPY in: hypothalamus, amygdala, caudate-putamen, hippocampus, and frontal cortex were evaluated. Single housed animals spent significantly more time on the open arms of the elevated plus maze and in the central area of the open field as compared to pair housed controls. These data are most likely indicative of enhanced exploration and novelty seeking. Concentrations of neuropeptide Y were increased in the caudate-putamen of the single housed subjects. NPY levels in caudate/putamen and hypothalamus were also significantly correlated with time spent in the open arms of the elevated plus maze. These data suggest that chronic social isolation, in these adult Wistar rats, did not increase anxiety but produced enhanced exploration in tests of anxiety, an effect that was associated with NPY concentrations in the striatum and hypothalamus.

Keywords: NPY; Anxiety; Animal models of depression; Caudate-putamen

1. Introduction

The use of chronic or repeated stressful events and/or social isolation/separation in rodents and primates have been used to model human affective disorders (see Koob et al., 1989). These models include restraint stress, cold water stress, exposure to dominant males, tail suspension, as well as maternal and peer separation/isolation (see Bowden and McKinney, 1972; Kalin, 1989; Ehlers et al., 1993; Hall, 1998; Bonilla-Jaime et al., 2003; Strekalova et al., 2004). Of these models, social isolation has proven to be highly consistent in its ability to produce increases in anxiety-like behavior (Parker and Morinan, 1986; Jankowska et al., 1991; Wright et al., 1991a,b; Maisonnette et al., 1993; Hall et al., 1998, 2000; Hall, 1998; Lodge and Lawrence, 2003; Hellemans et al., 2004; Weiss et al., 2004).

Neuropeptide Y (NPY; Tatemoto, 1982; Tatemoto et al., 1982) is one of the most abundantly expressed neuropeptides within the mammalian CNS and has been implicated in modulation of anxiety-related behaviors, stress-responses, depression, memory function, and regulation of feeding (for reviews see: Cerda-Reverter and Larhammar, 2000; Heilig and Thorsell, 2002; Chronwall and Zukowska, 2004; Kalra and Kalra, 2004; Levine et al., 2004; Thorsell and Ehlers, in press). The anti-anxiety effect of NPY has been demonstrated in a number of animal models (Broqua et al., 1995; Heilig et al., 1989), and the expression of NPY within the CNS has been shown to be regulated by external stressors (Thorsell et al., 1998, 1999). A possible involvement of central NPY in depression has been suggested in the literature (Jimenez-Vasquez et al., 2001). Electroconvulsive shock (ECS) treatment consistently upregulates brain NPY levels (Stenfors et al., 1989; Wahlestedt et al., 1990), while antidepressants have yielded inconsistent results (Heilig et al., 1988; Bellman and Sperk, 1993; Heilig and Ekman, 1995). NPY has been

* Corresponding author. Tel.: +1 858 784 7058; fax: +1 858 784 7475.
E-mail address: cindye@scripps.edu (C.L. Ehlers).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.12.005

demonstrated to be altered in the Flinders rat, an animal model of psychopathology (Caberlotto et al., 1998, 1999). Furthermore, NPY has been demonstrated to have an anti-depressant effect in the forced swim test (Redrobe et al., 2002).

Given the effects of NPY on anxiety and depression-like behaviors, we examined the effect of 12 weeks of adult social isolation, on exploratory and anxiety-related behavior as well as central NPY concentrations in Wistar rats. The hypothesis was that social isolation would lead to changes in central NPY concentrations that would be correlated with behavioral changes in the elevated plus maze and open field.

2. Material and methods

2.1. Subjects

Male Wistar rats ($n=20$; body weight 188±3 g, 45 days of age at beginning of experiment) were obtained from Charles River, USA. Upon arrival half of the animals were housed two per cage and the other half single housed. Animals were kept in a temperature and humidity controlled vivarium with a 12 h/12 h light/dark cycle (lights on at 6 AM). Animal care was in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Animals were randomly assigned to 2 groups upon arrival, single housed or pair housed. These housing conditions were maintained for 12 weeks. During this time the rats were only handled when weighed once per week, the behavioral paradigms were performed as described below. After 12 weeks, behavior in the elevated plus maze and open field was assessed. Half the animals were first exposed to the open field and then the elevated plus maze. For the remaining rats, the order of testing was reversed. This was done to eliminate any possible confounding factors that may be carried over from one test to the other. One week after testing was completed, the animals were then sacrificed and brains extracted for analysis of neuropeptide Y levels.

2.2. Elevated plus maze

Plus maze testing was conducted as previously described (Pellow et al., 1985; Pellow and File, 1986). The apparatus was made of black plastic and consisted of two open arms (50 × 10 cm), and two closed arms of the same size but with 40 cm high end and side walls. The arms were connected by a central 10 × 10 cm area. Testing was performed under dim red light with the maze elevated 50 cm above the white floor. To avoid effects of abrupt background noises a low white noise was presented at all times before, during, and after testing. Animals were adapted to the testing room for 1 h prior to testing. Rats were placed in the central area of the maze facing one of the open arms. The variables assessed included the total number of arm entries, percent of open arm entries, and percent time spent in the open arms during the 5 min test. An arm entry was defined as placement of all four paws into an arm.

2.3. Open field testing

Open field testing was performed without prior exposure to the apparatus as previously described (Slawecki and Roth, 2004). The open field was 76 cm (w) × 76 cm (l) × 50 cm (h). The floor of the open field was demarcated into 25 equally sized squares (16 perimeter squares and 9 center squares). During testing, the field was illuminated at a level of 50 lx by a single white light. On the test day, all subjects were weighed and transferred to a dimly lit ante-room 1 h before testing. At the start of each 5 min test, the subject was placed in the center of the open field. Variables assessed included: the latency to move to the perimeter, the percent of time spent in the center, and total square entries, and percent center square entries. Rearing and grooming in the open field was also recorded. The apparatus was cleaned with alcohol and water between each test.

2.4. Analysis of peptide levels

The following brain regions were dissected by a modification of the techniques of Glowinski and Iversen (1966), and Palkovits and Brownstein (1988): the hypothalamus, amygdala, the hippocampus, the frontal cortex and caudate-putamen. The tissue samples were weighed and frozen on dry-ice. Samples were stored at −80 °C until assayed for peptide content (Stenfors et al., 1989; Mathe et al., 1990a,b, 1997).

2.5. Statistical analysis

Statistical analysis was performed using Sigmaplot (Systat Inc.). Behavioral data were analyzed using one-way ANOVA. Body weight changes over the weeks of isolation housing were assessed using two-way ANOVAs [housing × week]. Housing was assessed as a between subjects factor. Week was assessed as a within subjects factor. Post hoc analyses used Tukey tests. One-way ANOVA was used to assess the effects of housing on brain peptide concentrations. Pearson correlation analyses were also performed to examine the possible relationship of peptide concentrations and behavioral measures. A level of $p<0.05$ was set as the criterion for statistical significance.

3. Results

3.1. Effects of housing condition on body weight

Significant changes in body weights were seen over the course of the experiment [$F(1,198)=570.18$, $p<0.001$]. Over the 12-week course of the experiment, both groups of rats gained weight at a steady rate. However, no differences in body weight were seen as a function of housing and no housing × weeks in isolation interactions were seen. At the time of behavioral testing, pair housed rats weighed an average of 533±22 g and single housed rats weighed an average of

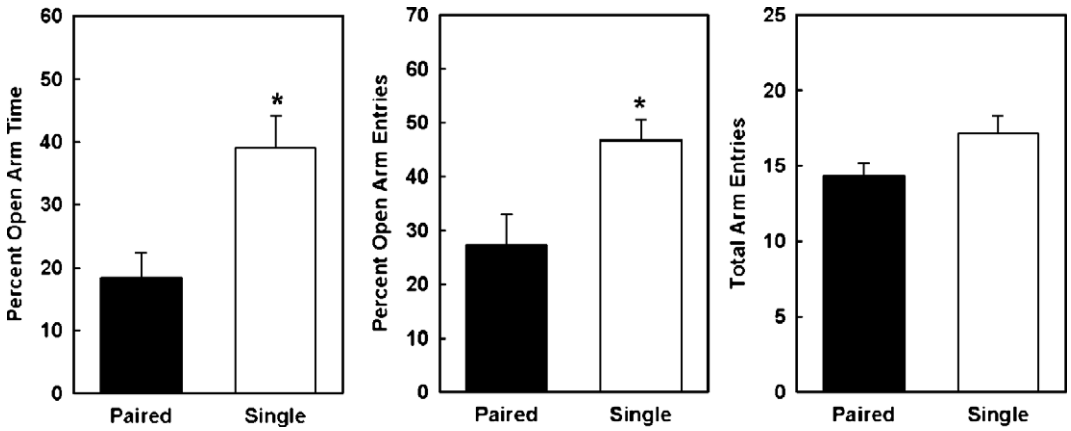

Fig. 1. Behavior in the elevated plus maze as a function of housing condition. Values are given as mean ± SEM. Pair housed (black bars, $n=9$). Single housed (white bars, $n=7$). Asterisk indicates significant difference between groups ($p<0.05$).

493 ± 13 g. On the day brain tissue was harvested for NPY analysis, pair housed rats weighed an average of 535 ± 22 g and single housed rats weighed an average of 519 ± 14 g. Although there were no significant differences in body weight between the experimental groups, the fact that the single housed rats generally weighed less is opposite to what has been previously found in this model (Ehlers et al., 1989).

3.2. Effects of housing condition on elevated plus maze behavior

Data from 4 rats (1 pair housed, 3 single housed) were omitted from the statistical analysis of behavior in the elevated plus maze due errors in data collection. Assessment of behavior in the elevated plus maze revealed significant effects of housing on percent time spent in the open arms [$F(1,15)=10.4$, $p=0.006$] and number of entries into the open arms [$F(1,15)=7.39$, $p=0.017$], but not for total arm entries [$F(1,15)=3.8$, $p=0.072$]. As is shown in Fig. 1, single housed rats spent more time in the open arms and made more entries into the open arms than did pair housed rats. Increased percent open arm time in the single housed group occurred in concert with significant decreases in percent closed arm time [$F(1,15)=11.5$, $p=0.004$; single housed = 43.6% ± 4.7% vs. pair housed = 68.1% ± 5.7%]. There were no group differences in percent of time spent in the center of the maze (single housed = 17.4% ± 1.5% vs. pair housed = 13.5% ± 1.9%).

3.3. Effects of housing condition on open field behavior

Data from 1 pair housed rat was omitted from the data analysis due to an error in data collection. Assessment of behavior in the open field revealed significant effects of housing on percent time spent in the center [$F(1,18)=8.17$, $p=0.011$], percent center square entries [$F(1,18)=11.24$, $p=0.004$], and grooming [$F(1,18)=13.61$, $p=0.002$]. As can be seen in Fig. 2, single housed rats spent more time in the center of the open field and made a greater percentage of center square entries. In addition, levels of grooming were elevated in the single housed rats (1.33 ± 0.31) relative to pair housed rats (0.2 ± 0.14). There were no effects of *housing* on total square entries or rearing in the open field.

3.4. Effects of housing condition on NPY levels

A summary of NPY levels in each of the brain regions are presented in Table 1. Statistical assessment of NPY levels revealed significant effects of *housing* in the caudate/putamen

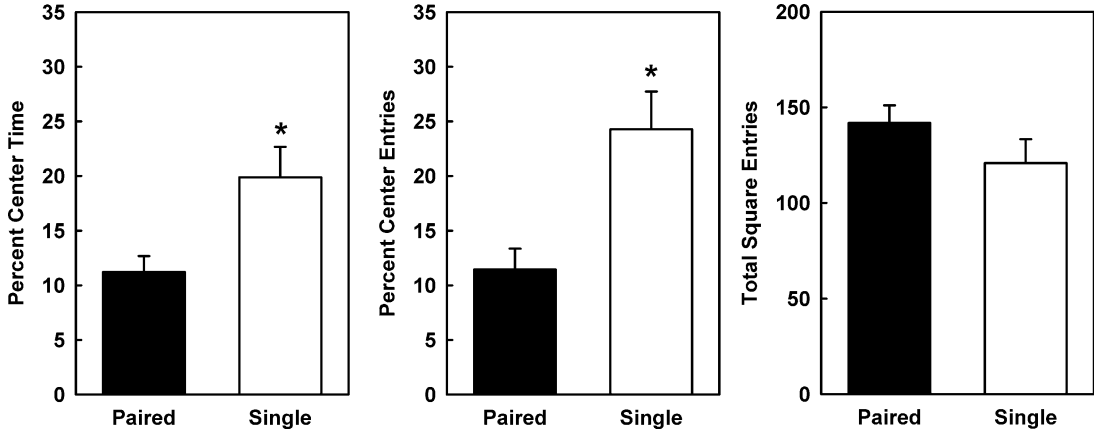

Fig. 2. Behavior in the open field as a function of housing condition. Values are given as mean ± SEM. Pair housed (black bars, $n=10$). Single housed (white bars, $n=9$). Asterisk indicates significant difference between groups ($p<0.05$).

Table 1
NPY levels in the brain as a function of housing condition

Region	Pair housed (pmol/g tissue)	Single housed (pmol/g tissue)
Amygdala	65±6	75±8
Caudate-putamen	19±3	32±6*
Frontal cortex	51±2	57±5
Hippocampus	13±3	12±2
Hypothalamus	225±21	257±29

Values are given as mean±SEM (n=10/group).
Asterisk indicates significant difference between groups (p<0.05).

[$F(1,19)=4.52$, 0.048], but not in any other brain region. Statistically significant correlations between NPY levels and elevated plus maze behavior were also found. A significant positive correlation was observed between NPY in the caudate/putamen and percent time spent in the open arms [Pearson correlation coefficient=0.532, p=0.034]. A significant positive correlation was also found between NPY in the hypothalamus and percent open arm entries [Pearson correlation coefficient=0.511, p=0.043]. There were no significant correlations between NPY levels in any of the brain regions assessed and behaviors in the open field.

4. Discussion

Social separation/isolation from either maternal or peer influence can induce a biobehavioral response in rodents and nonhuman primates seeming to mimic certain aspects of human psychopathology. For instance, the behavioral effects of social isolation in rats can include: enduring hyperactivity (Sahakian et al., 1974; Weinstock et al., 1976; Garzon and Del Rio, 1981; Ehlers et al., 1989) aggressiveness (Valzelli and Bernassconi, 1976; Day et al., 1982; Wongwitdecha and Marsden, 1996), enhanced stereotypy to amphetamine (Jones et al., 1990) impairment of performance (Morgan et al., 1977; File, 1978; Jones et al., 1989, 1991), deficits in prepulse inhibition of startle (Wilkinson et al., 1994), and neuroendocrine changes (Ehlers et al., 1993; Heidbreder et al., 2000). The nature and severity of these effects depend on the age at isolation and the strain of rat, and the specific testing conditions (for review see Hall, 1998).

In the present study, isolation housing in adult male Wistar rats was found to increase time spent in the open arms of the plus maze as well as time spent in the center of the open field. These types of changes are often interpreted as decreases in anxiety-like behavior, as rodents tend to avoid brightly lit open areas. Although some studies have not found increases in anxiety-like behavior following isolate housing (Wongwitdecha and Marsden, 1996;Vale and Montgomery, 1997), most studies have (Parker and Morinan, 1986; Jankowska et al., 1991; Wright et al., 1991a,b; Maisonnette et al., 1993; Hall et al., 1998, 2000; Hall, 1998; Lodge and Lawrence, 2003; Hellemans et al., 2004; Weiss et al., 2004). Enhanced anxiety also appears to be an enduring property of social isolation as resocialization of isolation-reared rats for a month does not reverse their anxiogenic profile (Wright et al., 1991a,b). Therefore, it is likely that the enhanced exploration of the plus maze and open field, found in the present study, may have an alternative explanation other than reduced anxiety.

An increase in the time spent in the center of the open field could be interpreted purely as an increase in exploration of a novel environment independent of whether the animal was "anxious" or not. A number of previous studies have found an increased in open field exploration in isolate housed rats (Syme, 1973; Einon et al., 1975; Gentsch et al., 1981; Plaznik et al., 1993; Fone et al., 1996; Lapiz et al., 2000; Arakawa, 2003). For example, Wistar rats that were isolate housed starting on postnatal day 51 were reported to spend more time in and make more entries into the center of the open field (Arakawa, 2003). Social deprivation has also been reported to enhance preference for a novel environment that was not aversive (Sahakian et al., 1977; Hall et al., 1997). Therefore, one explanation for the present set of findings is that social isolation enhances both exploratory tendencies and anxiety, behaviors are contrary to each other. Thus isolation exposed rats may display either anxiety or exploration depending on the conditions of the task (see Hall, 1998). Taken together, the most parsimonious explanation of the current data is that the conditions of the behavioral testing, in these isolation housed Wistar rats, favored enhancements of exploration and/or novelty-seeking behaviors rather than anxiety.

The neurochemical correlates of the behavioral consequences of isolation rearing/housing of rats are complex and involve many neurotransmitters, including the dopaminergic, opiate, glutaminergic, cholinergic and serotonergic systems (Schenk et al., 1982; Oehler et al., 1987; Jones et al., 1992; Hall, 1998; Hall et al., 1999, 2002; Dalley et al., 2002; Preece et al., 2004; Lehmann et al., 2004). However, few studies have evaluated peptide responses following isolation rearing/housing. In two animal models of depression, the Flinders Sensitive Line and the Fawn Hooded rats, NPY-LI was found to be decreased in the hippocampus (Mathe et al., 1998; Jimenez-Vasquez et al., 2000). Maternal separation has also been shown to produce lower levels of NPY-LI in the hippocampus and increased levels in the hypothalamus of maternally separated Sprague–Dawley rats (Jimenez-Vasquez et al., 2001). In the present study adult social isolation was found to produce increased levels of NPY in caudate-putamen, but not in the amygdala, that significantly correlated with behavior in the elevated plus maze. NPY levels in the amygdala have been consistently associated with anxiety (Heilig, 1995). Therefore, changes in NPY in the caudate-putamen, found in the present study, could theoretically be associated more specifically with the impact of isolation housing on motor behavior and exploration, rather than anxiety.

There is a large body of literature that supports the notion that exposure to isolation has a substantial impact on brain serotonin systems, particularly in striatum (see Hall, 1998 for review). For instance, isolation rearing has previously been reported to lead to significantly increased 5-HT fiber densities

in the dorsal part of the caudate-putamen (Lehmann et al., 2003). A diminished response of single neuron activity to iontophoretic application of serotonin in the striatum has also been demonstrated in rats after 3 months of social isolation (Oehler et al., 1987). Reduced presynaptic neuronal function to release 5-HT has also been reported in isolation-reared rats using microdialysis techniques (Bickerdike et al., 1993). Additionally 5,7-dihydroxytryptamine (5,7-DHT) injected into the anterior raphe nuclei has been found to produce an 80% decrease in striatal 5-HT concentrations, concomitant with a significant decrease in the number of NPY immunoreactive cells identified. These results suggest that a complete interruption of 5-HT transmission can lead to a decrease in the intracellular NPY level, which could be associated with a decrease in the release of the peptide. The authors further postulated that serotonergic neurons normally exert a positive influence on NPY striatal neurons (Compan et al., 1996). It has been also shown that expression of the serotonin [5-hydroxytryptamine (5-HT)] synthesis enzyme tryptophan hydroxylase is significantly reduced in mice that are deficient in the NPY Y1-receptor (Karl et al., 2004). Taken together these studies suggest that isolation induced decreases in serotonergic tone in striatum could result in striatal changes in NPY levels observed in the present study. However, such changes may not be straightforward as Hall et al. (1999) have demonstrated that variations in brain levels of serotonin resulting from seotonergic depetion have non-linear effects on serotonin release and behavior, and presumably also NPY levels. They have demonstrated that moderately depleted levels of serotonin produce increased anxiety whereas more severe depletion is associated with either normal or decreased levels of anxiety. These results may also partially explain why isolate animals may display anxiety or exploration in some behavioral tests. Taken together these studies suggest that social isolation may have important influences on anxiety, exploratory behavior and NPY levels in striatum.

Acknowledgements

This work is supported by a grant from National Institute of Alcohol Abuse and Alcoholism: RO1 AA 06059 to C.L. Ehlers and by the Swedish Medical Research grant 10414 and the Karolinska Institute to A.A. Mathe.

References

Arakawa H. The effects of isolation rearing on open-field behavior in male rats depends on developmental stages. Dev Psychobiol 2003;43:11–9.

Bellman R, Sperk G. Effects of antidepressant drug treatment on levels of NPY or prepro-NPY-mRNA in the rat brain. Neurochem Int 1993;22: 183–7.

Bickerdike MJ, Wright IK, Marsden CA. Social isolation attenuates rat forebrain 5-HT release induced by KCl stimulation and exposure to a novel environment. Behav Pharmacol 1993;4:231–6.

Bonilla-Jaime H, Retana-Marquez S, Vazquez-Placois G, Velazquez-Moctezuma J. Corticosterone and testosterone levels after chronic stress in an animal model of depression. Neuropsychobiology 2003;48:55–8.

Bowden DM, McKinney WT. Behavioral effects of peer separation, isolation, and reunion on adolescent male rhesus monkeys. Dev Psychobiol 1972; 5:353–62.

Broqua P, Wettstein JG, Rocher MN, Gauthier-Martin B, Junien JL. Behavioral effects of neuropeptide Y receptor agonists in the elevated plus-maze and fear-potentiated startle procedures. Behav Pharmacol 1995;6:215–22.

Caberlotto L, Fuxe K, Overstreet DH, Gerrard P, Hurd YL. Alterations in neuropeptide Y and Y1 receptor mRNA expression in brains from an animal model of depression: region specific adaptation after fluxetine treatment. Brain Res Mol Brain Res 1998;59:58–65.

Caberlotto L, Jimenez PA, Overstreet DH, Hurd YL, Mathe AA, Fuxe K. Alterations in the neuropeptide Y levels and Y1 bindings sites in the Flinders Sensitive rats, a genetic animal model of depression. Neurosci Lett 1999;265:191–4.

Cerda-Reverter JM, Larhammar D. Neuropeptide Y family of peptides: structure, anatomical expression, function, and molecular evolution. Biochem Cell Biol 2000;78:371–92.

Chronwall BM, Zukowska Z. Neuropeptide Y, ubiquitous and elusive. Peptides 2004;25:359–63.

Compan V, Dusticier N, Nieoullon A, Daszuta A. Opposite changes in striatal neuropeptide Y immunoreactivity after partial and complete serotonergic depletion in the rat. Synapse 1996;24:87–96.

Dalley JW, Theobald DE, Pereira EA, Li PM, Robbins TW. Specific abnormalities in serotonin release in the prefrontal cortex of isolation-reared rats measured during behavioural performance of a task assessing visual spatial attention and impulsivity. Psychopharmacology 2002;164: 329–40.

Day HD, Seay BM, Hale P, Hendricks D. Early social deprivation and the ontogeny of unrestricted social behavior in the laboratory rat. Dev Psychobiol 1982;15:47–59.

Ehlers CL, Wall TL, Wyss SP, Chaplin RI. Social zeitgebers: a peer separation model of depression in rats. In: Koob G, Ehlers CL, Kupfer DJ, editors. Animal models of depression. Boston: Birkhauser; 1989. p. 99–110.

Ehlers CL, Kaneko WM, Owens MJ, Nemeroff CB. Effects of gender and social isolation on electroencephalogram and neuroendocrine parameters in rats. Biol Psychiatry 1993;33:358–66.

Einon D, Margan MJ, Sahakian BJ. The development of intersession habituation and emergence in socially reared and isolated rats. Dev Psychobiol 1975;8:553–9.

File SE. Exploration, distraction, and habituation in rats reared in isolation. Dev Psychobiol 1978;11:73–81.

Fone KCF, Shalders K, Fox ZD, Arthur R, Marsden CA. Increased 5-HT_{2c} receptor responsiveness occurs on rearing rats in social isolation. Psychopharmacology 1996;123:346–52.

Garzon J, Del Rio J. Hyperactivity induced in rats by long-term isolation: further studies on a new animal model for the detection of antidepressants. Eur J Pharmacol 1981;74:278–94.

Gentsch C, Lichtsteiner M, Feer H. Locomotor activity, defecation score and corticosterone levels during an open-field exposure: a comparison among individually and group-housed rats, and genetically selected rat lines. Physiol Behav 1981;27:183–6.

Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain I The disposition of [3H]-norepinephrine, [3H]-dopamine, and [3H]-DOPA in various regions of the brain. J Neurochem 1966;13:665–9.

Hall FS. Social deprivation of neonatal, adolescent, and adult rats has distinct neuro-chemical and behavioral consequences. Crit Rev Neurobiol 1998; 1,2:129–62.

Hall FS, Humby T, Wilkinson LS, Robbins TW. The effects of isolation-rearing on preference by rats for a novel environment. Physiol Behav 1997; 62:299–303.

Hall FS, Huang S, Fong GW, Pert A, Linnoila M. Effects of isolation-rearing on locomotion, anxiety and responses to ethanol in Fawn Hooded and Wistar rats. Psychopharmacology 1998;139:203–9.

Hall FS, Wilkinson LS, Humby T, Robbins TW. Maternal deprivation of neonatal rats produces enduring changes in dopamine function. Synapse 1999;32:37–43.

Hall FS, Huang S, Fong GW, Sundstrom JM, Pert A. Differential basis of strain and rearing effects on open-field behavior in Fawn Hooded and Wistar rats. Physiol Behav 2000;71:525–32.

Hall FS, Ghaed S, Pert A, Xing G. The effects of isolation rearing on glutamate receptor NMDAR1A mRNA expression determined by in situ

hybridization in Fawn Hooded and Wistar rats. Pharmacol Biochem Behav 2002;73:185–91.
Heidbreder CA, Weiss IC, Domeney AM, Pryce C, Homberg J, Hedou G, et al. Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. Neuroscience 2000;100:749–68.
Heilig M. Antisense inhibition of neuropeptide Y (NPY)-Y1 receptor expression blocks the anxiolytic-like action of NPY in amygdala and paradoxically increases feeding. Regul Pept 1995;59:201–5.
Heilig M, Ekman R. Chronic parental antidepressant treatment in rats: unaltered levels and processing of neuropeptide Y (NPY) and corticotrophin-releasing hormone (CRH). Neurochem Int 1995;26:351–5.
Heilig M, Thorsell A. Brain neuropeptide Y (NPY) in stress and alcohol dependence. Rev Neurosci 2002;13:85–94.
Heilig M, Wahlstedt C, Ekman R, Widerlov E. Antidepressant drugs increase the concentration of neuropeptide Y (NPY)-like immunoreactivity in the rat brain. Eur J Pharmacol 1988;147:465–7.
Heilig M, Soderpalm B, Engle JA, Widerlov E. Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. Psychopharmacology 1989;98:524–9.
Hellemans KGC, Benge LC, Olmstead MC. Adolescent enrichment partially reverses the social isolation syndrome. Dev Brain Res 2004;150:103–15.
Institute for Laboratory Animal Resources. Guide for the care and use of laboratory animals. Washington DC: National Academy Press; 1996.
Jankowska E, Pucilowski O, Kostowski W. Chronic oral treatment with diltiazem or verapamil decreases isolation-induced activity impairment in elevated plus maze. Behav Brain Res 1991;43:155–8.
Jimenez-Vasquez PA, Overstreet DH, Mathe AA. Neuropeptide Y in male and female brains of Flinders Sensitive Line, a rat model of depression Effects of electroconvulsive stimuli. J Psychiatr Res 2000;34:405–12.
Jimenez-Vasquez PA, Mathe AA, Thomas JD, Riley EP, Ehlers CL. Early maternal deprivation alters neuropeptide Y concentrations in selected brain regions in adult rats. Dev Brain Res 2001;131:149–52.
Jones GH, Robbins TW, Marsden CA. Isolation rearing retards the development of schedule-induced polydipsia in rats. Physiol Behav 1989;45:71–8.
Jones GH, Marsden CA, Robbins TW. Increased sensitivity to amphetamine and reward-related stimuli following social isolation in rats: possible disruption of dopamine-dependent mechanism of the nucleus accumbens. Psychopharmacology 1990;102:364–72.
Jones GH, Marsden CA, Robbins TW. Behavioral rigidity and rule-learning deficits following isolation-rearing in the rat neurochemical correlates. Behav Brain Res 1991;43:35–50.
Jones GH, Hernandez TD, Kendall DA, Marsden CA, Robbins TW. Dopaminergic and serotonergic function following isolation rearing in rats: study of behavioural responses and postmortem and in vivo neurochemistry. Pharmacol Biochem Behav 1992;43:17–35.
Kalin NH. The HPA system and neuroendocrine models of depression. In: Koob GF, Ehlers CL, Kupfer DG, editors. Animal models of depression. Boston: Birkhauser; 1989. p. 57–73.
Kalra SP, Kalra PS. NPY and cohorts in regulating appetite, obesity and metabolic syndrome: beneficial effects of gene therapy. Neuropeptides 2004;38:201–11.
Karl T, Lin S, Schwarzer C, Sainsbury A, Couzens M, Wittmann W, et al. Y1 receptors regulate aggressive behavior by modulating serotonin pathways. Proc Natl Acad Sci USA 2004;101:12742–7.
Koob GF, Ehlers CL, Kupfer DJ. Animal models of depression. Boston: Birkhauser; 1989.
Lapiz MDS, Mateo Y, Parker T, Marsden C. Effects of noradrenaline depletion in the brain on response to novelty in isolation-reared rats. Psychopharmacology 2000;152:312–20.
Lehmann K, Lesting J, Polascheck D, Teuchert-Noodt G. Serotonin fibre densities in subcortical areas: differential effects of isolated rearing and methamphetamine. Brain Res Dev Brain Res 2003;147:143–52.
Lehmann K, Hundsdorfer B, Hartmann T, Teuchert-Noodt G. The acetylcholine fiber density of the neocortex is altered by isolated rearing and early methamphetamine intoxication in rodents. Exp Neurol 2004;189:131–40.
Levine AS, Jewett DC, Cleary JP, Kotz CM, Billington CJ. Our journey with neuropeptide Y: effects on ingestive behaviors and energy expenditure. Peptides 2004;25:505–10.
Lodge DJ, Lawrence AJ. The effect of isolation rearing on volitional ethanol consumption and central CCK/dopamine systems in Fawn Hooded rats. Behav Brain Res 2003;141:113–22.
Maisonnette S, Morato S, Brandao ML. Role of resocialization and of 5HT1A receptor activation on the anxiogenic effects induced by isolation in the elevated plus-maze test. Physiol Behav 1993;54:573–8.
Mathe AA, Jousisto-Hanson J, Stenfors C, Theodorsson E. Effect of lithium on tachykinins, calcitonin gene-related peptide, and neuropeptide Y in rat brain. J Neurosci Res 1990;26:233–7.
Mathe AA, Stenfors C, Brodin E, Theodorsson E. Neuropeptides in brain: effect of microwave irradiation and decapitation. Life Sci 1990;46:287–93.
Mathe AA, Gruber S, Jimenez P, Theodorsson E, Stenfors C. Effects of electroconvulsive stimuli and MK-801 on neuropeptide Y, neurokinin A, and calcitonin gene-related peptide in rat brain. Neurochem Res 1997;22:629–36.
Mathe AA, Jimenez P, Theodorsson E, Stenfors C. Neuropeptide Y, neurokinin A and Neurotensin in brain regions of Fawn Hooded 'depressed' Wistar, and Sprague–Dawley rats Effects of electroconvulsive stimuli. Prog Neuropsychopharmacol Biol Psychiatry 1998;22:529–46.
Morgan MJ, Einon D, Morris RG. Inhibition and isolation rearing in the rat: extinction and satiation. Physiol Behav 1977;18:1–5.
Oehler J, Jahkel M, Schmidt J. Neuronal transmitter sensitivity after social isolation in rats. Physiol Behav 1987;41:187–91.
Palkovits M, Brownstein MJ. Maps and guide to microdissection of the rat brain. Amsterdam, Holland: Elsevier Press; 1988.
Parker V, Morinan A. The socially isolated rat as a model for anxiety. Neuropharmacology 1986;25:663.
Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 1986;24:525–9.
Pellow S, Chopin P, File SE, Briley M. Validation of open-closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.
Plaznik A, Palejko W, Stefanski R, Kostowski W. Open field behavior of rats reared in different social conditions: the effects of stress in imipramine. Pol J Pharmacol 1993;45:243–52.
Preece MA, Dalley JW, Theobald DE, Robbins TW, Reynolds GP. Region specific changes in forebrain 5-hydroxytrptamine 1A and 5-hydroxytrptamine 2A receptors in isolation-reared rats: an in vitro autoradiography study. Neuroscience 2004;123:725–32.
Redrobe JP, Dumont Y, Fournier A, Quirion R. The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. Neuropsychopharmacology 2002;26:124–615.
Sahakian BJ, Robbins TW, Morgan M, Iversen SD. The effects of psychomotor stimulants on sterotype and locomotor activity in socially-deprived and control rats. Brain Res 1974;84:195–205.
Sahakian BJ, Robbins TW, Iversen SD. The effects of isolation rearing on exploration in the rat. Anim Learn Behav 1977;5:193–8.
Schenk S, Britt MD, Atalay J, Charleston S. Isolation rearing decreases opiate receptor binding in rat brain. Pharmacol Biochem Behav 1982;16:841–2.
Slawecki CJ, Roth J. Comparison of the onset of hypoactivity and anxiety-like behavior during alcohol withdrawal in adolescent and adult rats. Alcohol Clin Exp Res 2004;28:598–607.
Strekalova T, Spanagel R, Bartsch D, Henn FA, Gass P. Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. Neuropsycopharmacology 2004;29:2007–17.
Stenfors C, Theodorsson E, Mathe AA. Effect of repeated eloctroconvulsive treatment of regional concentrations of tachykinins, neurotensin, vasoactive interstinal polypeptide, neuropeptide Y, and galanin in rat brain. J Neurosci Res 1989;24:445–50.
Syme LA. Social isolation at weaning: some effects on two measures of activity. Anim Learn Behav 1973;1:161–3.
Tatemoto K. Neuropeptide Y: complete amino acid sequence of the brain peptide. Proc Natl Acad Sci USA 1982;79:5485–9.

Tatemoto K, Carlquist M, Mutt V. Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 1982;296:659–60.

Thorsell A, Ehlers C.L. Neuropeptide Y (NPY) in brain function. In: Handbook of neurochemistry and neuroscience. In press.

Thorsell A, Svensson P, Wiklund L, Sommer W, Ekman R, Heilig M. Suppressed neuropeptide Y (NPY) mRNA in rat amygdala following restraint stress. Regul Pept 1998;75–76:247–54.

Thorsell A, Carlsson K, Ekman R, Heilig M. Behavioral and endocrine adaptation, and up-regulation of NPY expression in rat amygdala following repeated restraint stress. Neuroreport 1999;10:3003–7.

Vale AL, Montgomery AMJ. Social interaction: responses to chlordiazepoxide and the loss of isolation-reared effects with paired-housing. Psychopharmacology 1997;133:127–32.

Valzelli L, Bernassconi S. Psychoactive drug effect on behavioral changes induced by prolonged socio-environmental deprivation in rats. Phys Med Biol 1976;6:271–6.

Wahlestedt C, Blendy JA, Kellar KJ, Heilig M, Widerlov E, Ekman R. Electroconvulsive shocks increase the concentration of neocortical and hippocampal neuropeptide Y (NPY)-like immunoreactivity in the rat. Brain Res 1990;507:65–8.

Weinstock M, Spiser Z, Ashkenazi R. Biochemical and pharmacological studies on an animal model of hyperactivity states. In: Gershon E, Belmarker R, Kety S, Rosenbaum M, editors. The impact of biology on modern psychiatry. New York: Plenum Press; 1976.

Weiss IC, Pryce CR, Jongen-Rêlo AL, Nanz-Bohr NI, Feldon J. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res 2004;152:279–95.

Wilkinson LS, Killcross SS, Humby T, Hall FS, Geyer MA, Robbins TW. Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. Neuropsychopharmacology 1994;10:61–72.

Wongwitdecha N, Marsden CA. Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. Behav Brain Res 1996;75:27–32.

Wright IK, Ismail H, Upton N, Marsden CA. Effect of isolation rearing on 5-HT agonist-induced responses in the rat. Psychopharmacology 1991;105:259–63.

Wright IK, Upton N, Marsden CA. Resocialisation of isolation-reared rats does not alter their anxiogenic profile on the elevated X-maze model of anxiety. Physiol Behav 1991;50:1129–32.