Pharmacology, Biochemistry and Behavior 96 (2010) 59–66

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Effects of fluoxetine and PCPA on isolation-induced morphine self-administration and startle reactivity

S. Raz [a,*], B.D. Berger [b]

[a] The Center for Psychobiological Research, Department of Psychology, The Academic College of Emek Yezreel, Emek Yezreel 19300, Israel
[b] Department of Psychology, University of Haifa. Mount Carmel, Haifa, 31905, Israel

ARTICLE INFO

Article history:
Received 5 March 2009
Received in revised form 29 November 2009
Accepted 12 April 2010
Available online 18 April 2010

Keywords:
Social isolation
Morphine
Self-administration
Startle response
Fluoxetine
PCPA
Serotonin

ABSTRACT

The present study investigated the effects of the SSRI fluoxetine and the serotonin synthesis blocker — parachlorophenylalanine (PCPA) on morphine self-administration and startle reactivity in rats subjected to social isolation during adulthood. Adult Wistar rats were housed individually or in pairs for 21 days. They were treated with fluoxetine, PCPA, or vehicle and tested for their startle response and intake of a morphine solution (0.5 mg/ml). Socially restricted rats consumed significantly more morphine solution (but not water) than rats living in pairs, in both one-bottle and in two-bottle tests. They also showed significantly higher startle response amplitude. Daily fluoxetine treatment (5 mg/kg i.p.) counteracted these behavioral alterations induced by isolation housing while PCPA treatment (200 mg/kg for 3 consecutive days) further exacerbated it. Social isolation may increase morphine self-administration and emotional reactivity in the startle box by affecting serotonin. Antidepressants (such as fluoxetine) may normalize or stabilize serotonin function and restore the behavioral changes produced by isolation.

1. Introduction

Clinical studies in humans indicate that social stressors such as social isolation, lack of supporting social network, or antisocial personality disorder are risk factors for hypertension, coronary heart disease, poorer outcomes and increased mortality after life-threatening events, as well as for emotional disorders including stress, anxiety, depression and suicide attempts (Boden-Albala et al., 2005; Cacioppo and Hawkley, 2003; Duberstein et al., 2004; Hawthorne, 2008; Kessler, 1997; Mookadam and Arthur, 2004; Oetzel et al., 2007; Stockdale et al., 2007; Tennant, 1999). Moreover, social isolation is viewed as a major etiological factor in the development of compulsive drug abuse, more chronic and severe addiction, and higher rates of dropouts and relapse after withdrawal attempts (Compton et al., 2003, 2005; Darke et al., 2005; Dobkin et al., 2002; Oetzel et al., 2007; Pelissier and O'Neil, 2000; Stockdale et al., 2007; Westermeyer and Thuras, 2005). Such environmental and situational factors associated with drug use can interact with the behavioral, subjective, and rewarding effects of a given drug, thus influencing the propensity to use the same drug again (Caprioli et al., 2007). It follows that comprehensive laboratory models of drug seeking behavior should include these components.

Preventing rats from normal interaction and communication affects physiological and behavioral processes (Brain and Benton, 1979; Hall, 1998; Valzelli and Garattini, 1972). Many studies have focused on the long-term consequences of isolation at infancy or just before weaning (isolation rearing). In some studies rats were isolated during both weaning and adulthood (isolated housing) confounding whether the resultant behavioral changes could be attributed to isolated rearing, isolated housing or an interaction between these states. A relatively small number of studies have examined social isolation during adulthood (for review see Hall, 1998; Lu et al., 2003).

Individual housing increases aggression and interferes with the performance of a cooperation task in male rats (Byrd and Briner, 1999; Miachon et al., 2000; Rilke et al., 2001; Sanchez and Meier, 1997; Schuster et al., 1993; Swanson and Schuster, 1987; Vale and Montgomery, 1997; Valzelli, 1971; Willner et al., 1989; Wongwitdecha and Marsden, 1996). Rats housed individually tend to be more irritable, restless and hyperactive compared with rats housed in groups (Bakshi and Geyer, 1999; Domeney and Feldon, 1998; Wilkinson et al., 1994). They also show patterns of hypersensitivity, novelty-seeking, anxiety, stress and depressive-like behavior (Brenes et al., 2008; Brenes Saenz et al., 2008; Grippo et al., 2007; Hall et al., 1997; Nunes Mamede Rosa et al., 2005; Robbins, 1992; Serra et al., 2000; Sudakov et al., 2003; Weiss et al., 2004; Westenbroek et al., 2005; Whitaker-Azmitia et al., 2000; Willner, 1984). Isolation also affects physiological parameters such as: hyperfunction of the HPA axis, elevated levels of plasma corticosterone, heavier adrenal glands, increased heart rate, and hypertension (Gadek-Michalska et al., 1994; Grippo et al., 2007; Nagaraja and Jeganathan, 1999; Serra et al., 2000; Stranahan et al., 2006; Weiss et al., 2004; Westenbroek et al., 2005; Wright and Ingenito, 2003).

* Corresponding author. Tel.: +972 546634301; fax: +972 46423618.
E-mail address: Sivanr@yvc.ac.il (S. Raz).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.04.007

Social isolation has also been shown to affect psychoactive drug self-administration, though the results of these studies are not consistent, perhaps due to variations in experimental design, drug delivery system, type of drug, dosage, etc (Caprioli et al., 2007). However, several studies have reported that exposure of rats to environmental and social stressors, correlate with enhanced tendency to consume psychostimulants, opiates and alcohol (for review see Lu et al., 2003; Stairs and Bardo, 2009(. Morphine has been shown to be effective in reversing isolation effects on various behavioral and physiological parameters (Bardo et al., 1997; Hol et al., 1996; Jimenez and Fuentes, 1993; Sudakov et al., 2003; Van den Berg et al., 1999; Van den Berg et al., 2000). Several studies have shown that rats housed in isolation tend to consume higher amounts of morphine and other opiates compared with grouped housed rats (Alexander et al., 1978; Alexander et al., 1981; Heyne, 1996; Shaham et al., 1992). We have reported that adult rats housed in isolation self administer significantly higher amounts of morphine solution (but not water) compared with rats housed in pairs (Raz and Berger, 2010).

Social isolation is associated with alterations in multiple brain structures and functions, particularly with changes in serotonin function (Hall, 1998; Hall et al., 1998; Fone and Porkess, 2008; Robbins et al., 1996; Serra et al., 2007; Whitaker-Azmitia et al., 2000). In general, isolation-rearing and isolation-housing of rats has been found to decrease serotonin concentration, disrupt serotonin synthesis and release and alter serotonin turnover in prefrontal cortex, hippocampus and nucleus accumbens (Brenes et al., 2008; Brenes and Fornaguera, 2009; Dalley et al., 2002; Fone and Porkess, 2008; Hall, 1998; Hall et al., 1998; Heibreder et al., 2000; Jones et al., 1992; Muchimapura et al., 2002; Muchimapura et al., 2003; Parker and Morinan, 1986; Preece et al., 2004; Robbins et al., 1996; Segal et al., 1973; Whitaker-Azmitia et al., 2000). Further support, though indirect, for serotonergic changes subsequent to isolation-housing involve the ability of some antidepressant drugs especially those acting to enhance or to balance serotonin activity (i.e. fluoxetine, fluvoxamine, sertraline, femoxetine, anpirtoline, fluprazine and imipramine) to reverse isolation-induced aggression, sucrose consumption, immobility behavior, impaired coping, cognitive deficits, anxiety and cooperation deficits (Berger and Schuster, 1987; Brenes and Fornaguera, 2009; Greco et al., 1990; Heritch et al., 1990; Maisonnette et al., 1993; Olivier et al., 1989; Ramanathan et al., 2003; Rilke et al., 2001; Ruedi-Bettschen et al., 2004; Sanchez and Hyttel, 1994; Sanchez and Meier, 1997; Willner, 1984).

Fluoxetine was the first selective serotonin re-uptake inhibitor to be widely available for treatment of depression and other neuropsychiatric disorders. It has been also suggested as a treatment for drug abuse and dependence though with mixed results.

In rats, fluoxetine has been found to change or reverse some of the neuronal and behavioral effects of morphine including**:** oral stereotypy (Wennemer and Kornetsky, 1999); morphine sensitization (Sills and Fletcher, 1997); and neuronal hyperactivity (Akaoka and Aston-Jones, 1993). However, while there is some evidence for the attenuating effect of fluoxetine on alcohol and cocaine self administration in rats (Glatz et al., 2002; Homberg et al., 2004; Le et al., 1999), there is, to our knowledge, no evidence for the effect of fluoxetine on opiates and especially on morphine self administration in socially isolated rats.

The aim of the present study is to further investigate the relationship between social isolation at adulthood, morphine self administration, startle response and drug manipulations that affect serotonin. Therefore, we examine the effect of the SSRI fluoxetine (Exp. 1) and of the serotonin synthesis blocker, parachlorophenylalanine (PCPA) (Exp. 2) on startle reactivity and morphine self-administration of isolation-housed vs. paired-housed rats. We hypothesize that fluoxetine would reduce startle response amplitude and intake of morphine solution self-administration and that PCPA would further enhance these behaviors in socially restricted rats.

2. Materials and methods

2.1. Subjects

Subjects were adult male Wistar rats (Harlan). Their age at the beginning of the experiments was 56 days and their average weight was 210 g. Throughout the study, subjects were kept in room controlled for temperature (23 ± 1 °C) and maintained on a 12-hour light/dark cycle (lights on — 0700 h) in standard cages with transparent walls and sawdust bedding. Water and other solutions were given through external bottles hanging on the cage. Daily fluid consumption was measured by weighing bottles before presentation and again after 24 h. All procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and approved by the institutional ethics Committee. Morphine sulfate was obtained from Rafa Laboratories, Jerusalem. Fluoxetine hydrochloride and parachlorophenylalanine (PCPA) were obtained from Sigma-Aldrich.

2.2. Experimental procedures

Upon arrival**,** animals were housed 6 per cage and allowed to adapt to the animal facility for 1 week.

2.2.1. Stage I — adaptation (Days 1–21)

Rats were assigned randomly to the different experimental groups. In both experiments**,** subjects were first divided in two groups of differing housing conditions: Isolated housing: 1 animal per 40 × 25 × 18 cm cage ($n = 20$) and Social housing: 2 animals per 56 × 34 × 19 cm cage ($n = 20$). Each group then subdivided again in two to create 4 ($n = 10$) groups.

In Experiment 1, half the animals (10 isolated and 10 paired) were treated with fluoxetine hydrochloride (5 mg/kg dissolved in distilled water in a volume of 2.5 ml/kg, IP) and half (10 isolated and 10 paired) were treated with vehicle (distilled water at 2.5 ml/kg). Fluoxetine or vehicle treatment was given daily, starting after 7 days of adaptation to housing conditions and lasting throughout the experiment (Days 8–34).

In Experiment 2, half (10 isolated and 10 paired) were treated with PCPA (200 mg/kg dissolved in saline 0.9% in a volume of 4 ml/kg, IP) and half (10 isolated and 10 paired) with saline (at 4 ml/kg). PCPA or saline treatment was given acutely in three consecutive days on Days 16–18 of the adaptation phase. On Days 19–21, rats were given the chance to recover before the starting of behavioral tests. This acute PCPA treatment has been found to result in up to 90% depletion of brain serotonin (Koe and Weissman, 1966; Richter-Levin and Segal, 1989).

Animals in both experiments were maintained under these different housing conditions for 21 days with access to food and water *ad libitum*. Water intake was measured during the last 3 days of this phase (Days 19–21). Behavioral tests began on Day 22.

2.2.2. Stage II — startle response (Day 22)

Startle response was measured using an automated, ventilated, sound-attenuated JR. startle box (Hamilton-Kinder, USA) that was positioned in a dimly lighted room. The startle box consisted of a Plexiglas chamber mounted on a piezoelectric accelerometer. Movements of the rats inside the chamber resulted in changes of the voltage output of the accelerometer. These signals were amplified, digitized, and fed into a data-acquisition board in a computer for further analysis. Rats were habituated for 30 min to the startle test room before being placed in the chamber. The startle session started with a 5-min acclimatization period, with a background noise level of 57 dB which was maintained throughout the session. Rats were subjected to 10 tones (40 ms, 110 dB noise stimulus) with intervals of 1 min. The maximum startle response for each trial was measured by

arbitrary units (AU). The average of the 10 responses of each animal was taken as an index of the intensity of its startle reflex response.

2.2.3. Stage III — forced morphine consumption (one-bottle test) (Days 23–28)

Subjects were given access to Morphine sulfate solution only (0.5 mg/ml) for 6 days. Morphine solution intake was measured every 24 h by weighing the bottles. Rats in the social housing were provided with one bottle. So as to obtain an estimate of the morphine consumption for a single animal, the total intake was divided in half.

2.2.4. Stage IV — choice test (two-bottle test) (Days 29–34)

Subjects were given access to both water and morphine solution (0.5 mg/ml) for 6 days. Again, intake amounts were estimated by weighing bottles every 24 h and total intake of paired housed rats was divided in two.

2.3. Statistical analysis

For the startle response test, data were analyzed using One-Way ANOVA followed by post-hoc Tukey tests (HSD). For the morphine consumption tests, data were analyzed by ANOVA for repeated measures (mixed design). Housing condition (isolated vs. paired rats) and treatment (fluoxetine or PCPA vs. vehicle) was assessed as between-group factors and day was assessed as within-subject factor. Tukey (HSD) was used as the post hoc test when appropriate. For pairs of rats, the best estimate of the intake for a single animal was taken as the mean intake of the pair. To reduce statistical bias, we considered each pair as a single animal for analysis. Numeric results are presented as Mean ± SEM (in both text and figures) and considered significant for p-values less than 0.05.

We assumed that 1 g equals 1 ml, and therefore we present morphine solution and water consumption in ml units.

3. Results

3.1. Experiment 1

Effects of fluoxetine on startle response amplitude and morphine self-administration of isolated vs. paired housed rats.

3.1.1. Weight

Throughout the experiment no significant differences in body weight were seen as a function of housing conditions or fluoxetine treatment (Table 1). Data are presented as total volume of morphine solution or water consumed. "Mean" represents the average morphine solution or water consumption of all days of measurements.

3.1.2. Startle response

Fluoxetine treatment resulted in significant reduction of mean startle amplitude of isolated rats. There were significant differences between groups [$F(3,36) = 4.83$, $p<0.006$]. Post hoc analysis revealed that the mean startle response of isolated rats treated with vehicle was higher (1374.7 ± 352.98) relative to paired rats treated with vehicle (950.4 ± 325.06) (HSD, $p<0.04$). However, Isolated rats treated with fluoxetine exhibited lower levels of startle reactivity (871.05 ± 349.93) compared with isolated rats treated with vehicle (1374.7 ± 352.98) (HSD, $p<0.01$). No differences were found between isolated rats treated with fluoxetine and paired rats treated with either fluoxetine or vehicle (Fig. 1a).

3.1.3. Morphine self-administration

3.1.3.1. Forced test (one-bottle test). Significant differences in morphine solution intake were seen between groups [$F(3,26) = 5.63$, $p<0.004$]. The days*group interaction was not significant. Isolated rats treated with vehicle consumed higher amounts of morphine solution (16.54 ± 1.21) compared with paired rats treated with vehicle (10.53 ± 1.5) (HSD, $p<0.02$) or fluoxetine (9.68 ± 1.03) (HSD, $p<0.01$). Isolated rats treated with fluoxetine consumed significantly lower amounts of morphine solution (11.77 ± 1.31) relative to isolates treated with vehicle (16.59 ± 1.21) (HSD, $p<0.03$). No differences were found between isolates treated with fluoxetine and paired animals treated with either fluoxetine or vehicle (Fig. 1b).

3.1.3.2. Choice test (two-bottle test). Significant differences in morphine solution intake were seen between groups [$F(3,26) = 67.1$, $p<0.0001$]. A significant days*group interaction was also found [$F(15) = 4.2$, $p<0.0001$]. Isolated rats treated with vehicle self-administered higher amounts of morphine solution (11.35 ± 0.21) than paired housed rats treated with vehicle (7.78 ± 0.29) (HSD, $p<0.0001$) or fluoxetine (7.78 ± 0.29) (HSD, $p<0.0001$). Isolates treated with fluoxetine consumed lower amounts of morphine solution (8.38 ± 0.16) than isolates treated with vehicle (11.35 ± 0.21) (HSD, $p<0.0001$). There were no differences in morphine intake between isolates treated with fluoxetine and pairs treated with either fluoxetine or vehicle. No differences in water intake were seen between all 4 groups (Fig. 1c).

3.2. Experiment 2

Effects of PCPA on startle response amplitude and morphine self-administration of isolated vs. paired housed rats.

3.2.1. Weight

Throughout the experiment, no significant differences in body weight were seen as a function of housing conditions. PCPA treatment resulted in decreased body weight of isolates as well as paired rats compared with control rats [$F(3,36)=20.14$, $p<0.0001$]. However, those differences "disappeared" on the third day of the forced morphine test (Day 24) and from then on remained similar in all groups (Table 2).

3.2.2. Startle response

PCPA treatment resulted in significant enhancement of mean startle amplitude of isolated and paired housed rats. There were significant differences between groups [$F(3,36) = 14.87$, $p<0.0001$]. Post hoc analysis revealed that the mean startle response of isolated rats treated with saline was higher (1098 ± 241.59) relative to paired rats treated with saline (814.32 ± 204.54) (HSD, $p<0.02$). However, Isolated rats treated with PCPA exhibited higher levels of startle reactivity (1421.164.41) compared with isolated rats treated with

Table 1
Body weight development during the duration of Experiment 1.

Time point in study	Isolation-vehicle	Isolation-fluoxetine	Pairs-vehicle	Pairs-fluoxetine
Day of housing (Day 1)	208.4 ± 4.37	209.2 ± 3.8	211.7 ± 4.52	214 ± 5.19
First day of fluoxetine/vehicle treatment (Day 8)	254 ± 4	252.3 ± 4.21	259.7 ± 4.59	261.4 ± 4.25
Day of startle response test, after 14 days of fluoxetine/vehicle treatment (Day 22)	307.8 ± 6.73	301.7 ± 6.73	320 ± 3.44	302.2 ± 5.65
First day of morphine/water choice test (Day 29)	294.1 ± 13.1	280.3 ± 9.75	301.9 ± 9.12	286.1 ± 7.82
End of study (Day 34)	317.9 ± 7.6	310.9 ± 8.16	329.8 ± 5.03	306.2 ± 6.32

Values are given in grams as mean ± SEM.

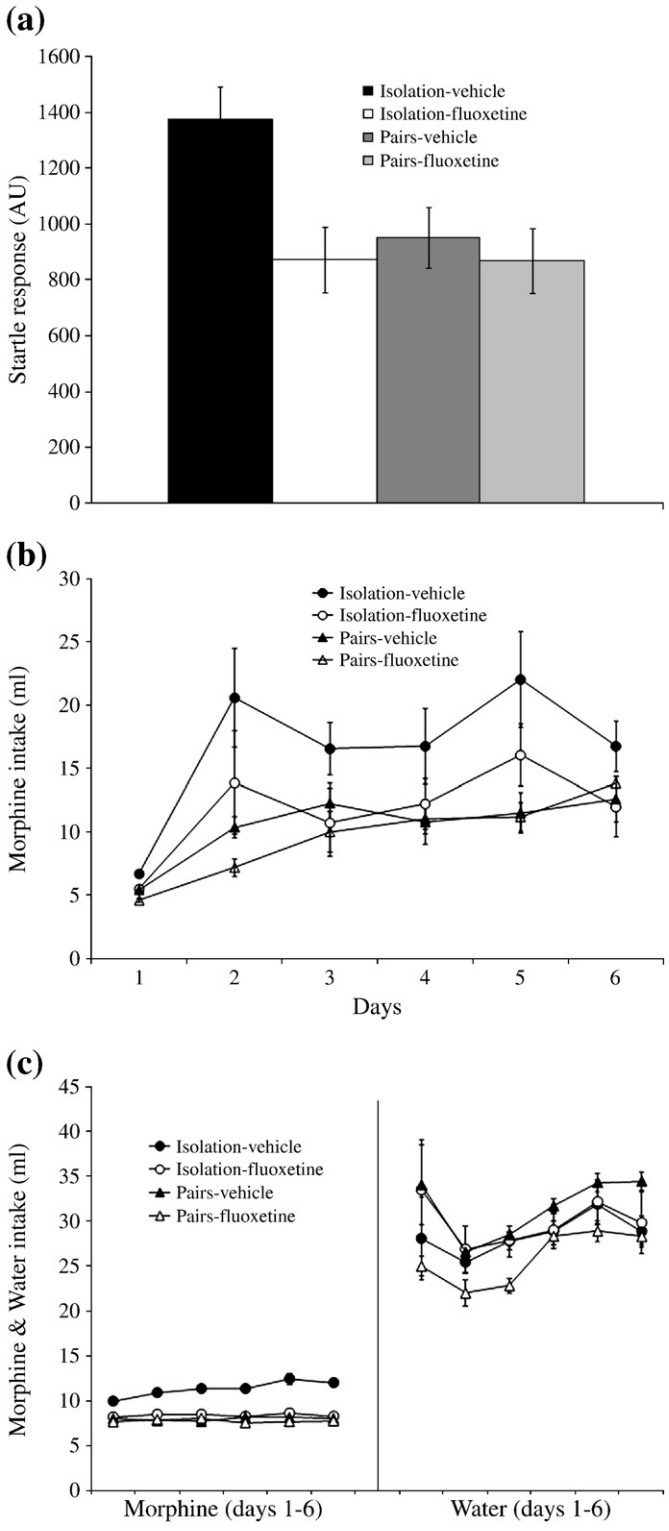

Fig. 1. Mean startle response amplitude (in arbitrary units) (a); Morphine solution intake (ml) during 6 days of one-bottle test (b); Morphine solution and water intake (ml) during 6 days of two-bottle test (c), among isolated vs. paired rats treated with fluoxetine or vehicle. Error bars represent SEM.

saline (1098 ± 241.59) (HSD, $p<0.009$). Paired rats treated with PCPA exhibited higher levels of startle reactivity (1277.62 ± 238.43) compared with pairs treated with saline (814.32 ± 204.54) (HSD, $p<0.0001$). No differences were found between isolated rats treated with PCPA and paired rats treated with PCPA (Fig. 2a).

3.2.3. Morphine self-administration

3.2.3.1. Forced test (one-bottle test). Significant differences in morphine solution intake were seen between groups [$F(3,26)=14.98$, $p<0.0001$]. The days*group interaction was not significant. Isolated rats treated with saline consumed higher amounts of morphine solution (12.38 ± 0.76) compared with pairs treated with saline (6.82 ± 0.89) (HSD, $p<0.05$). Isolated rats treated with PCPA consumed significantly higher amounts of morphine solution (18.19 ± 1.55) relative to isolates treated with saline (12.38 ± 0.76) (HSD, $p<0.01$) and to pairs treated with saline (6.82 ± 0.89) (HSD, $p<0.0001$). Pairs treated with PCPA consumed higher amounts of morphine solution (20.94 ± 2.34) compared with pairs treated with saline (6.82 ± 0.89) (HSD, $p<0.0001$) and compared with isolates treated with saline (12.38 ± 0.76) (HSD, $p<0.002$). No differences were found between isolates treated with PCPA and paired animals treated with PCPA (Fig. 2b).

3.2.3.2. Choice test (two-bottle test). Again there were significant differences in morphine solution intake between groups [$F(3,26)=10.1$, $p<0.0001$]. A days*group interaction was found [$F(15)=2.83$, $p<0.001$]. Isolated rats treated with saline self-administered higher amounts of morphine solution (10.18 ± 0.4) than paired housed rats treated with saline (6.84 ± 0.32) (this finding reached marginal statistic significance; HSD, $p<0.06$). Isolates treated with PCPA consumed higher amounts of morphine solution (13.29 ± 0.83) than isolates treated with saline (10.18 ± 0.4) (HSD, $p<0.03$) and than pairs treated with saline (6.84 ± 0.32) (HSD, $p<0.0001$). Pairs treated with PCPA consumed more morphine solution (13.07 ± 1.78) compared with pairs treated with saline (6.84 ± 0.32) (HSD, $p<0.001$). There were no differences in morphine intake between isolates treated with PCPA and pairs treated with PCPA. There were no differences in water intake between all 4 groups (Fig. 2c).

4. Discussion

The major aim of the present study was to investigate the effects of the SSRI fluoxetine and the serotonin synthesis blocker, PCPA, on morphine self-administration and on the startle response of rats subjected to social isolation during adulthood.

In keeping with earlier studies (Raz and Berger, 2010), social isolation in adult rats increased morphine intake relative to socially housed control groups. In addition, isolated housing augmented a non-conditioned startle response.

The SSRI, fluoxetine, counteracted these behavioral alterations induced by isolation housing while the 5HT synthesis inhibitor, PCPA, further exacerbated them. Rats in isolation treated with fluoxetine self-administered lower amounts of morphine and had lower startle response compared with isolates treated with vehicle and had similar morphine intake as paired rats. Isolates treated with PCPA self-administered higher amounts of morphine and had higher startle response compared with isolates treated with saline. Paired rats treated with PCPA consumed much more of the drug and had much higher startle response than paired rats treated with saline.

In laboratory rats, isolation-rearing and isolation-housing have been reported to decrease serotonin concentration, alter turnover, and disrupt presynaptic serotonin activity (release and synthesis) in several brain areas (Bickerdike et al., 1993; Brenes et al., 2008; Brenes and Fornaguera, 2009; Dalley et al., 2002; Fone and Porkess, 2008; Hall, 1998; Hall et al., 1998; Heibreder et al., 2000; Jones et al., 1992; Lapiz et al., 2003; Miura et al., 2005; Muchimapura et al., 2002; Muchimapura et al., 2003; Parker and Morinan, 1986; Preece et al., 2004; Robbins et al., 1996; Segal et al., 1973; Whitaker-Azmitia et al., 2000). These effects of social isolation on serotonin function vary as a function of age, period of isolation, duration of isolation, and the brain region studied.

Table 2
Body weight development during the duration of experiment 2.

Time point in study	Isolation-saline	Isolation-PCPA	Pairs-saline	Pairs-PCPA
Day of housing (Day 1)	227.3 ± 1.95	228.2 ± 2.14	229 ± 1.93	224.4 ± 2.35
First day of PCPA/saline treatment (Day 16)	283.1 ± 3.95	284.8 ± 4.19	287.2 ± 3.07	284.3 ± 3.69
Day of startle response test, after 3 recovery days from PCPA (Day 22)	304.8 ± 4.86	275.8 ± 5.44	310.7 ± 3.12	274.5 ± 2.94
First day of morphine/water choice test (Day 29)	248.1 ± 12.13	253.5 ± 9.44	256.3 ± 11.52	239.9 ± 11.54
End of study (Day 34)	269 ± 12.75	277.7 ± 8.24	283.8 ± 12.39	265.7 ± 10.88

Values are given in grams as mean ± SEM.

The majority of such studies use "isolation rearing" in which rats are housed individually from a relatively young age (mostly starting on post natal days 21–28). There are fewer studies in which isolated housing is initiated during adulthood (for review see Hall, 1998). In addition, there are a small number of studies comparing individually housed versus socially housed adult rats on opiate self-administration and especially morphine self-administration of (Alexander et al., 1978; Alexander et al., 1981). Finally, to our knowledge, there are no reports examining the effects of SSRI's and of PCPA on morphine self-administration or startle reactivity of isolated adult rats.

Startle response is a common behavioral test for assessment of emotional reactivity in rodents, and is often used to assess the effects of anti-anxiety drugs (Bourin et al., 2007; Grillon, 2002; Grillon, 2008; Rodgers, 1997). In trying to understand the putative mechanisms underlying the effects of fluoxetine and PCPA on startle response in the present study, it is important to differentiate between conditioned and unconditioned startle responses. Many studies have focused on fear-conditioning or fear-potentiated startle paradigm. In this model, the animals are trained to associate a neutral stimulus with an aversive stimulus and hence, after a few pairings, the CS induces a state of fear as measured by a potentiation of the startle response. The suggested neuronal basis of the fear-potentiated startle involves primarily the amygdaloid complex and its connections with some other rostral brain structures (reviewed by Davis et al., 1993 and by Koch, 1999). However, a growing number of studies suggest that fear-potentiated startle reflects a rapid conditioned response to the fear provoking stimulus, and does not provide an ideal model for more general and durable states of stress, anxiety, discomfort and apprehension (Davis et al., 1997; Gewirtz et al., 1998; Grillon and Baas, 2003; Koch, 1999). In the present study, no conditioning was used so the startle reflex was basically an unconditioned response. In this case, the startle response is thought to be mediated by a relatively simple neuronal circuit located in the lower brain stem including, among others, the caudal pontine reticular nucleus and the dorsal periaqueductal gray (Brandao et al., 2008; Koch, 1999). It has been also suggested that another structure may play an important role in the startle-enhancing effects of more durable states of stress and anxiety — the bed nucleus of the stria terminalis (BNST) (Davis et al., 1997; Gewirtz et al., 1998; Grillon and Baas, 2003; Koch, 1999). In view of this, it is possible that those lower brain stem structures or the BNST, rather than the amygdala, were the main neural substrates for the action of fluoxetine in counteracting (and of PCPA exacerbating) the effects of isolation-potentiated startle seen in the present study.

A commonly held view is that in the rat and in other species isolated housing and restricted social interaction produce physiological and behavioral changes that may be catalogued under the general category of "stress". Indeed, social isolation in laboratory animals has been suggested as a model for stress, anxiety and depression in humans (Brenes et al., 2008; Brenes et al., 2009; Cryan and Holmes, 2005; Cryan and Slattery, 2007; Fone and Porkess, 2008; Fuchs and Fliugge, 2006; Harris, 1989; Heidbreder et al., 2000; Katz, 1981; McKinney, 1984; Pryce et al., 2005; Thorsell et al., 2006; Willner, 1984). Consumption of morphine in isolated rats may be seen as "self-medication", hypothetically bringing relief from the unpleasant state of stress. Since socially housed rats are not exposed to the stressful consequences of social isolation, they do not benefit in the same

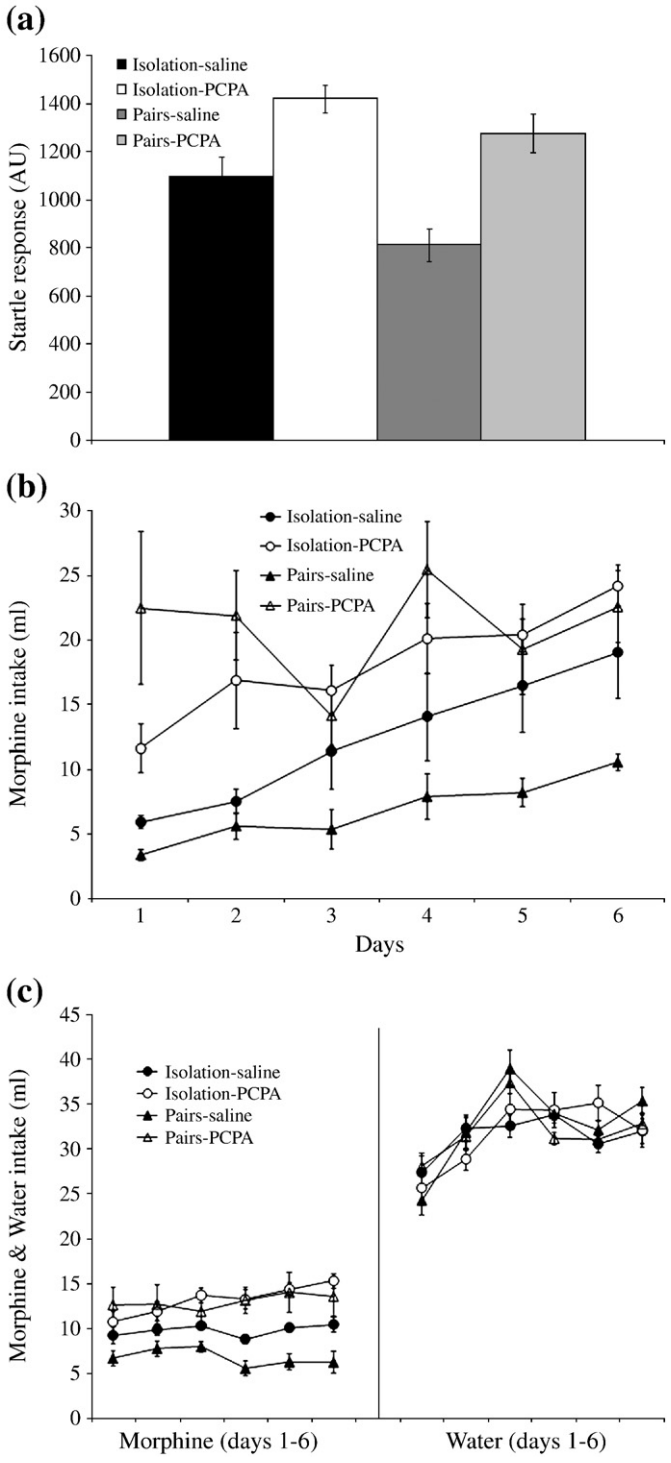

Fig. 2. Mean startle response amplitude (in arbitrary units) (a); Morphine solution intake (ml) during 6 days of one-bottle test (b); Morphine solution and water intake (ml) during 6 days of two-bottle test (c), among isolated vs. paired rats treated with PCPA or saline. Error bars represent SEM.

manner from morphine consumption which might even interfere with the performance of normal social interaction and therefore is consumed at lower doses (Alexander et al., 1978, 1981; McIntosh et al., 1980; Panksepp et al., 1979).

Opioid systems are involved in the regulation of social behaviors in juvenile and adult rats and several lines of evidence suggest an involvement of opioid systems in changes associated with social isolation. Social interaction has been found to induce release of opioid peptides in several brain areas, suggesting that isolation might reduce release of endogenous opioid peptides (Benton and Brain, 1988; Hol et al., 1996; Panksepp et al., 1980; Petkov et al., 1985; Schenk et al., 1987; Van den Berg et al., 1999). These authors postulate that exogenous opioid treatment during the isolation period might substitute for social interaction-induced endogenous opioid peptide release. Indeed, it has been observed that morphine treatment in rats subjected to isolation increases adult social activity and enhances opioid peptide release as compared with saline treated rats. Both social activity and opioid peptide release were unaffected by morphine treatment in non-isolated rats (Van den Berg et al., 1999).

There are other possible explanations for the effects of social isolation on morphine intake (Raz and Berger, 2010). The fact that there were no differences in water intake between the groups suggests that this finding cannot be explained by a general enhancement of fluid intake by isolates, but rather due to selective enhancement in morphine intake. Moreover, since there were no significant differences in body weight between isolated vs. paired subjects during the course of experiments (see also Thorsell et al., 2005; Thorsell et al., 2006) these results cannot be explained by differences in body weight, that might affect daily intake and/or drug reactivity.

Social isolation may change the sensitivity and reactivity to various stimuli. Therefore isolated rats may be more or less reactive to the bitter taste of morphine or to the novelty of the taste as opposed to the psychoactive action of the drug. Indeed, in our laboratory we have preliminary data that isolation housing may increase the intake of a bitter solution of quinine. Supporting our assumption of a pharmacological action of the drug as the most appropriate explanation of the pattern of results in our studies, we have found in preliminary experiments that the opiate antagonist naltrexone reversed the increase in morphine consumption following isolated housing (Raz and Berger, 2005). Isolated rats treated daily with naltrexone (5 mg/kg), consume significantly lower amounts of morphine solution than isolated rats treated with saline and similar amounts as their socially housed counterparts. Since naltrexone is not known to affect taste reactivity (Arbisi et al., 1999; Goodwin et al., 2001; Scinska et al., 2000) it is perhaps more likely that it reduces drug intake of isolates because it attenuates the psychopharmacological action of morphine.

Changes in serotonin pathways following social restriction have been linked to the development of aggressive behavior, depression, anxiety and substance abuse in both humans and animals (Heinz et al., 2001; Tamashiro et al., 2005; Matsumoto et al., 2005; Wrase et al., 2006). Moreover, the therapeutic effects of SSRI drugs and some anti-anxiety treatments are often attributed to modulation of dysfunctional serotonergic systems (Berger and Schuster, 1987; Brenes and Fornaguera, 2009; Greco et al., 1990; Heritch et al., 1990; Maisonnette et al., 1993; Olivier et al., 1989; Ramanathan et al., 2003; Rilke et al., 2001; Ruedi-Bettschen et al., 2004; Sanchez and Hyttel, 1994; Sanchez and Meier, 1997; Willner, 1984).

Taken together, it therefore is reasonable to speculate that social isolation alters serotonergic function and underlies the behavioral changes such as higher tendency to self-administer morphine and higher emotional reactivity in the startle box. Antidepressants (such as fluoxetine) act to normalize or stabilize serotonin tone and function, and therefore restore the behavioral changes observed in isolated rats.

References

Akaoka H, Aston-Jones G. Indirect serotonergic agonists attenuate neuronal opiate withdrawal. Neuroscience 1993;54:561–5.

Alexander BK, Beyerstein BL, Hadaway PF, Combas RB. Effects of early and later colony housing on oral ingestion of morphine in rats. Pharmacol Biochem Behav 1981;15:571–6.

Alexander BK, Coambs RB, Hadaway PF. The effects of housing and gender on morphine self-administration in rats. Psychopharmacology 1978;58:175–9.

Arbisi PA, Billington CJ, Levine AS. The effect of naltrexone on taste detection and recognition threshold. Appetite 1999;32:241–9.

Bakshi VP, Geyer MA. Ontogeny of isolation rearing-induced deficits in sensorimotor gating in rats. Physiol Behav 1999;67:385–92.

Bardo MT, Robinet PM, Hammer Jr RF. Effects of differential rearing environments on morphine-induced behaviors, opioid receptors and dopamine synthesis. Neuropharmacology 1997;36:251–9.

Benton D, Brain PF. The role of opioid mechanisms in social interaction and attachment. In: Rodjers RJ, Cooper SJ, editors. Endorphins, opiates and behavioural processes. New York: Wiley; 1988. p. 217–35.

Berger BD, Schuster R. Pharmacological aspects of social cooperation. In: Olivier B, Mos J, Brain PF, editors. Ethopharmacology of agonistic behavior in animals and humans. Martinus Nijhoff publishers; 1987. p. 14–32.

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

Boden-Albala B, Litwak E, Elkind MS, Rundek T, Sacco RL. Social isolation and outcomes post stroke. Neurology 2005;64:1888–92.

Bourin M, Petit-Demoouliere B, Dhonnchadha BN, Hascoet M. Animal models of anxiety in mice. Fundam Clin Pharmacol 2007;21:567–74.

Brain P, Benton D. The interpretation of physiological correlates of differential housing in laboratory rats. Life Sci 1979;24:99-115.

Brandao ML, Zanoveli JM, Ruiz-Martinez RC, Oliviera LC, Landiera-Fernandez J. Different patterns of freezing behavior organized in the periaqueductal gray of rats: association with different types of anxiety. Behav Brain Res 2008;188:1-13.

Brenes JC, Fornaguera J. The effect of chronic fluoxetine on social isolation-induced changes on sucrose consumption, immobility behavior, and on serotonin and dopamine function in hippocampus and ventral striatum. Behav Brain Res 2009;198:199–205.

Brenes JC, Rodriguez O, Fornaguera J. Differential effect of environmental enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. Pharmacol Biochem Behav 2008;89:85–93.

Brenes JC, Padilla M, Fornaguera J. A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. Behav Brain Res 2009;197:125–37.

Brenes Sáenz JC, Villagra OR, Fornaguera Trías J. Factor analysis of forced swimming test, sucrose preference test and open field test on enriched, social and isolated reared rats. Behav Brain Res 2008;169:57–65.

Byrd R, Briner WE. Fighting, nonagonistic social behavior, and exploration in isolation-reared rats. Aggress Behav 1999;25:211–23.

Cacioppo JT, Hawkley LC. Social isolation and health, with an emphasis underlying mechanisms. Perspect Biol Med 2003;46:39–52.

Caprioli D, Celentano M, Paolone G, Badiani A. Modeling the role of environment in addiction. Prog Neuropsychopharmacol Biol Psychiatry 2007:1639–53.

Compton WM, Conway KP, Stinson FS, Colliver JD, Grant BF. Prevalence, correlates, and comorbidity of DSM-IV antisocial personality syndromes alcohol and specific drug use disorders in the United States: results from the national epidemiologic survey on alcohol and related conditions. J Clin Psychiatry 2005;66:677–85.

Compton W, Cottler B, Jacobs JL, Ben-Abdallah A, Spitznagel EL. The role of psychiatric disorders in predicting drug dependence treatment outcomes. Am J Psychiatry 2003;160:890–5.

Cryan JF, Slattery DA. Animal models of mood disorders: recent developments. Curr Opin Psychiatry 2007;20:1–7.

Cryan JF, Holmes A. The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 2005;4:775–90.

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

Darke S, Williamson A, Ross J, Teesson M. Attempted suicide among heroin users: 12-month outcomes from the Australian Treatment Outcome Study (ATOS). Drug Alcohol Depend 2005;78:177–86.

Davis M, Falls WA, Campeau S, Kim M. Fear-potentiated startle: a neural and pharmacological analysis. Behav Brain Res 1993;58:175–98.

Davis M, Walker DL, Lee Y. Amygdala and bed nucleus of the stria terminalis: differential roles in fear and anxiety measured with the acoustic startle reflex. Philos Trans R Soc Lond 1997;B352:1657–87.

Dobkin PL, De CM, Paraherakis A, Gill K. The role of functional social support in treatment retention and outcomes among outpatient adult substance abusers. Addiction 2002;97:347–56.

Domeney A, Feldon J. The disruption of prepulse inhibition by social isolation in the wistar rat: how robust is the effect? Pharmacol Biochem Behav 1998;59:883–90.

Duberstein PR, Conwell Y, Conner KR, Eberly S, Evinger JS, Caine ED. Poor social integration and suicide: fact or artifact? A case-control study. Psychol Med 2004;34:1331–7.

Fone KC, Porkess MV. Behavioural and neurochemical effects of post-weaning social isolation in rodents — relevance to developmental neuropsychiatric disorders. Neurosci Biobehav Rev 2008;32:1087–102.

Fuchs E, Fliugge G. Experimental animal models for the simulation of depression and anxiety. Dialogues Clin Neurosci 2006;8:323–33.
Gadek-Michalska A, Boryez J, Bugajski J. Effect of social isolation on corticosterone secretion elicited by histaminergic stimulation. Agents actions 1994;41:77–9.
Gewirtz JC, McNish KA, Davis M. Lesions of the bed nucleus of the stria terminalis block sensitization of the acoustic startle reflex produced by repeated stress, but not fear-potentiated startle. Prog Neuropsychopharmacol 1998;22:625–48.
Glatz AC, Ehrlich M, Bae RS, Clarke MJ, Quinlan PA, Brown EC, Rada P, Hoebel BG. Inhibition of cocain self-administration by fluoxetine or D-fenfluramine combined with phentermine. Pharmacol Biochem Behav 2002;71:197–204.
Goodwin FL, Campisi M, Babinska I, Amit Z. Effects of naltrexone on the intake of ethanol and flavored solutions in rats. Alcohol 2001;25:9-19.
Greco AM, Gambardella P, Stticchi R, D'Aponte D, De Franciscis P. Chronic administration of imipramine antagonizes deranged circadian rhythm phases in individually housed rats. Physiol Behav 1990;48:67–72.
Grillon C. Models and mechanisms of anxiety: evidence from startle studies. Psychopharmacology 2008;199:421–37.
Grillon C. Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. Biol Psychiatry 2002;52:958–75.
Grillon C, Baas j. A review of the modulation of the startle reflex by affective states and its application in psychiatry. Clin Neurophysiol 2003;114:1557–79.
Grippo AJ, Lamb DG, Carter CS, Porges SW. Social isolation disrupts autonomic regulation of the heart and influences negative affective behaviors. Biol Psychiatry 2007;62:1162–70.
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. Social deprivation of neonatal, adolescent and adult rats has distinct neurochemical and behavioral consequences. Crit Rev Neurobiol 1998;12:129–62.
Hall FS, Wilkinson LS, Humby T, Inglis W, Kendall DA, Marsden CA, Robbins TW. Isolation rearing in rats: pre-and postsynaptic changes in Striatal dopaminergic systems. Pharmacol Biochem Behav 1998;59:859–72.
Harris JC. Experimental animal modeling of depression and anxiety. Psychiatr Clin North Am 1989;12:815–36.
Hawthorne G. Perceived social isolation in a community sample: its prevalence and correlates with aspects of peoples' lives. Soc Psychiatry Psychiatr Epidemiol 2008;43:140–50.
Heidbreder CA, Weiss IC, Domeney AM, Pryce C, Homberg J, Hedou G, Feldon J, Moran MC, Nelson P. Behavioural, neurochemical and endocrinological characterization of the early social isolation syndrome. Neuroscience 2000;100: 749–68.
Heinz A, Mann K, Weinberger DR, Goldman D. Serotonergic dysfunction, negative mood states, and response to alcohol. Alcohol Clin Exp Res 2001;25:487–95.
Heritch AJ, Henderson K, Westfall TC. Effects of social isolation on brain catecholamines and forced swimming in rats: prevention by antidepressant treatment. J Psychiatr Res 1990;24:251–8.
Heyne A. The development of opiate addiction in the rat. Pharmacol Biochem Behav 1996;53:11–25.
Hol T, Ruven S, Van Ree JM, Spruijt BM. Chronic administration of Org2766 and morphine counteracts isolation-induced increase in social interest: Implication of endogenous opioid systems. Neuropeptides 1996;30:283–91.
Homberg JR, Arends B, Warden G, Rasso HS, Schoffelmeer AN, de Vries TJ. Individual differences in the effects of serotonergic anxiolytic drugs on the motivation to self-administer cocaine. Neuroscience 2004;128:121–30.
Jimenez I, Fuentes JA. Subchronic treatment with morphine inhibits the hypertension induced by isolation stress in the rat. Neuropharmacology 1993;32: 223–7.
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.
Katz RJ. Animal models and human depressive disorders. Neurosci Biobehav Rev 1981;5:231–46.
Kessler RC. The effects of stressful life events on depression. Annu Rev Psycho 1997;48: 191–214.
Koch M. The neurobiology of startle. Prog Neurobiol 1999;59:107–28.
Koe KB, Weissman A. p-chlorophenylalanine: a specific depletory of brain serotonin. J Pharmacol Exp Ther 1966;154:499–516.
Lapiz MD, Fulforf A, Muchimapura S, Mason R, Parker T, Marsden CA. Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neurosci Behav Physiol 2003;33:13–29.
Le AD, Poulos CX, Harding S, Watchus J, Juzytsch W, Shaham Y. Effects of naltrexone and fluoxetine on alcohol self-administration and reinstatement of alcohol seeking induced by priming injection of alcohol and exposure to stress. Neuropsychopharmacology 1999;21:435–44.
Lu L, Shepard JD, Hall FS, Shaham Y. Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neurosci Biobehav Rev 2003;27:457–91.
Miachon S, Jouvenet M, Vallon JJ. Cholesterol and triglyceride levels in the serum of muricidal male wistar rats: indices of mitochondrial benzodiazepine receptors? Brain Res Bull 2000;51:57–61.
Maisonnette S, Morato S, Brandao ML. Role of resocialization and of5-HT1A receptor activation on the anxiogenic effects induced by isolation in the elevated plus-maze test. Physiol Behav 1993;54:753–8.
Matsumoto K, Pinna G, Puia G, Guidotti A, Costa E. Social isolation stress-induced aggression in mice: a model to study the pharmacology of neurosteroidogenesis. Stress 2005;8:85–93.
McIntosh TK, Vallano ML, Barfield RJ. Effects of morphine, beta- endorphin and naloxone on catecholamine levels and sexual behavior in the male rat. Pharmacol Biochem Behav 1980;13:435–41.
McKinney WT. Animal models of depression: an overview. Psychiatr Dev 1984;2: 77–96.
Miura H, Qiao H, Kitagami T, Ohta T, Ozaki N. Effects of fluvoxamine on levels of dopamine, serotonin, and their metabolites in the hippocampus elicited by isolation housing and novelty stress in adult rats. Int J Neurosci 2005;115:367–78.
Mookadam F, Arthur HM. Social support and its relationship to morbidity and mortality after acute myocardial infarction: systematic overview. Arch Intern Med 2004;164: 1514–8.
Muchimapura S, Mason R, Marsden CA. Effect of isolation rearing on pre-and post-synaptic serotonergic function in the rat dorsal hippocampus. Synapse 2003;47: 209–17.
Muchimapura S, Fulford AJ, Mason R, Marsden CA. Isolation rearing in the rat disrupts the hippocampal response to stress. Neuroscience 2002;112:697–705.
Nagaraja HS, Jeganathan PS. Influence of different types of stress on selected cardiovascular parameters in rats. Indian J Physiol Pharmacol 1999;43:296–304.
Nunes Mamede Rosa ML, Nobre MJ, Ribeiro Oliveira A, Brandao ML. Isolation-induced changes in ultrasonic vocalization, fear-potentiated startle and prepulse inhibition in rats. Neuropsychobiology 2005;51:248–55.
Oetzel J, Duran B, Jiang Y, Lucero J. Social support and social undermining as correlates for alcohol, drug, and mental disorders in American Indian women presenting for primary care at an Indian health service hospital. J Health Commun 2007;12: 187–206.
Olivier B, Mos J, van der Heyden J, Hartog J. Serotonergic modulation of social interactions in isolated male mice. Psychopharmacology 1989;97:154–6.
Panksepp J, Herman BH, Vilberg T, Bishop P, DeEskinazi FG. Endogenous opioids and social behavior. Neurosci Biobehav Rev 1980;4:473–87.
Panksepp J, Najam N, Soares F. Morphine reduces social cohesion in rats. Pharmacol Biochem Behav 1979;11:131–4.
Parker V, Morinan A. The socially-isolated rat as a model for anxiety. Neuropharmacology 1986;25:663–4.
Pelissier BM, O'Neil JA. Antisocial personality and depression among incarcerated drug treatment participants. J Subst Abuse 2000;11:379–93.
Petkov VV, Konstantinova E, Grachovska T. Changes in brain opiate receptors in rats with isolation syndrome. Pharmacol Res Comm 1985;17:575–84.
Preece MA, Dalley JW, Theobald DE, Robbins TW, Reynolds GP. Region specific changes in forebrain 5-hydroxytryptamine1A and 5-hydroxytryptamine2A receptors in isolation-reared rats: an in vitro autoradiography study. Neuroscience 2004;123:725–32.
Pryce CR, Rüedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, Feldon J. Long-term effects of early-life environmental manipulations in rodents and primates: potential animal models in depression research. Neurosci Biobehav Rev 2005;29:649–74.
Ramanathan M, Kumar SN, Suresh B. Evaluation of cognitive function of fluoxetine, sertraline and tianeptine in isolation and chronic unpredictable mild stress-induced depressive Wistar rats. Indian J Exp Biol 2003;41:1269–72.
Raz S, Berger BD. Social isolation increases morphine intake: behavioral and psychopharmacological aspects. Behav Pharmacol 2010;39:21–46.
Raz S, Berger BD. Socially restricted housing in laboratory rats as a model for the study of drug seeking behavior in humans. Presented as poster at the ninth annual meeting of the Israel Society for Biological Psychiatry, Hagoshrim; 2005.
Richter-Levin G, Segal M. Spatial performance is severely impaired in rats with combined reduction of serotonergic and cholinergic transmission. Brain Res 1989;477:404–7.
Rilke O, Will K, Jaehkel M, Oehler J. Behavioral and neurochemical effects of anpirtoline and citalopram in isolated and group housed mice. Prog Neuropsychopharmacol Biol Psychiatry 2001;25:1125–44.
Robbins TW. Are animal models of mental illness viable. In: Elliott JM, Heal DJ, Marsden CA, editors. Experimental approaches to anxiety and depression. London: Jhon Wiley; 1992. p. 219–31.
Robbins TW, Jones GH, Wilkinson LS. Behavioural and neurochemical effects of early social deprivation in the rat. J Psychopharmacol 1996;10:39–47.
Rodgers RJ. Animal models of 'anxiety': where next? Behav Pharmacol 1997;8:477–96.
Ruedi-Bettschen D, Feldon J, Pryce CR. The impaired coping induced by early deprivation is reversed by chronic fluoxetine treatment in adult fischer rats. Behav Pharmacol 2004;15:413–21.
Sanchez C, Hyttel J. Isolation-induced aggression in mice: effects of 5- hydroxytryptamine uptake inhibitors and involvement of postsynaptic 5-HT1A receprors. Eur J Pharmacol 1994;264:241–7.
Sanchez C, Meier E. Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? Psychopharmacology 1997;129:197–205.
Schenk S, Hunt T, Klukowski G, Amit Z. Isolation housing decreases the effectiveness of morphine in the conditioned taste aversion paradigm. Psychopharmacology 1987;92:51–8.
Scinska A, Koros E, Polanowska E, Kukwa A, Bogucka-Bonikowska A, Kostowski W, Habrat B, Bienkowski P. An opioid receptor antagonist, naltrexone, does not alter taste and smell responses in humans. Poland J pharmacol 2000;52:397–402.
Schuster R, Berger BD, Swanson HH. Cooperative social coordination and aggression: II. Effects of sex and housing among three strains of intact laboratory rats differing in aggressiveness. Q J Exp Psychol(B) 1993;46:367–90.
Segal DS, Knapp S, Kuczenski RT, Mandell AJ. The effects of environmental isolation on behavior and regional rat brain tyrosine hydroxylase and tryptophan hydroxylase activities. Behav Biol 1973;8:47–53.
Serra M, Pisu MG, Mostallino MC, Sanna E, Biggio G. Changes in neuroactive steroid content during social isolation stress modulates GABAA receptor plasticity and function. Brain Res Rev 2007;57:520–30.

Serra M, Pisu MG, Littera M, Papi G, Sanna E, Tuveri F, Usala L, Purdy RH. Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(A) receptor function in rat brain. J Neurochem 2000;75:732–40.
Shaham Y, Alvares K, Nespor SM, Grunberg NE. Effects of stress on oral morphine and fentanyl self-administration in rats. Pharmacol Biochem Behav 1992;41:615–9.
Sills TL, Fletcher PJ. Fluoxetine attenuates morphine-induced locomotion and blocks morphine-sensitization. Eur J Pharmacol 1997;337:161–4.
Stairs DJ, Bardo MT. Neurobehavioral effects of environmental enrichment and drug abuse vulnerability. Pharmacol Biochem Behav 2009;92:377–82.
Stockdale SE, Wells KB, Tang L, Belin TR, Zhang L, Sherbourne CD. The importance of social context: neighborhood stressors, stress-buffering mechanisms, and alcohol, drug, and mental health disorders. Soc Sci Med 2007;65:1867–81.
Stranahan AM, Khalil D, Gould E. Social isolation delays the positive effects of running on adult neurogenesis. Nat Neurosci 2006;9:526–33.
Sudakov SK, Medvedeva OF, Rusakova IV, Figurina IB. Effect of social isolation on behavioral parameters and sensitivity of rats to morphine and caffeine. Bull Exp Biol Med 2003;135:11–3.
Swanson HH, Schuster R. Cooperative social coordination and aggression in male laboratory rats: effects of housing and testosterone. Horm Behav 1987;21:310–30.
Tamashiro KL, Nguyen MM, Sakai RR. Social stress: from rodents to primates. Front Neuroendocrinol 2005;26:27–40.
Tennant C. Life stress, social support and coronary heart disease. Aust NZ J Psychiatry 1999;33:636–41.
Thorsell A, Slawecki CJ, El Khoury A, Mathe AA, Ehlers CL. The effects of social isolation on neuropeptide Y levels, exploratory and anxiety-related behaviors in rats. Pharmacol Biochem Behav 2006;83:28–34.
Thorsell A, Slawecki CJ, El Khoury A, Mathe AA, Ehlers CL. Effects of social isolation on ethanol consumption and substance P/neurokinin expression in Wistar rats. Alcohol 2005;36:91–7.
Vale AL, Montgomery AM. Social interaction: responses to chlordiazepoxide and the loss of isolation-reared effects with paired-housing. Psychopharmacology 1997;133:127–32.
Valzelli L. Aggression in rats and mice, behavioral and biochemical aspects. Actual Pharmacol 1971;24:133–52.
Valzelli L, Garattini S. Biochemical and behavioural changes induced by isolation in rats. Neuropharmacology 1972;11:17–22.
Van den Berg CL, Van Ree JM, Spruijt BM. Morphine attenuates the effects of juvenile isolation in rats. Neuropharmacology 2000;39:969–76.
Van den Berg CL, Kitchen I, Gerrits MA, Spruijt BM, Van Ree JM. Morphine treatment during juvenile isolation increases social activity and opioid peptides release in the adult rat. Brain Res 1999;830:16–23.
Weiss IC, Pryce CR, Jongen-Relo AL, Nanz-Bahr NI, Feldon J. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res 2004;152:279–95.
Wennemer HK, Kornetsky C. Fluoxetine blocks expression but not development of sensitization to morphine- induced oral stereotypy in rats. Psychopharmacology 1999;146:19–23.
Westenbroek C, Snijders TAB, den Boer JA, Gerrits M, Fokkema DS, Ter Host GR. Pair-housing of male and female rats during chronic stress exposure results in gender-specific behavioral responses. Horm Behav 2005;47:620–8.
Westermeyer J, Thuras P. Association of antisocial personality disorder and substance disorder morbidity in a clinical sample. Am J Drug Alcohol Abuse 2005;31:93-110.
Whittaker-Azmitia P, Zhou F, Hobin J, Borella A. Isolation-rearing of rats produces deficit as adults in the serotonergic innervation of hippocampus. Peptides 2000;21: 1755–9.
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.
Willner P. The validity of animal models of depression. Psychopharmacol 1984;83:1-16.
Willner P, Sampson D, Phillips G, Fichera R, Foxlow P, Muscat R. Effects of isolated housing and chronic antidepressant treatment on cooperative social behaviour in rats. Behav Pharmacol 1989;1:85–90.
Wongwitdecha N, Marsden CA. Social isolation increases aggressive behavior and alters the effects of diazepam in the rat social interaction test. Behav Brain Res 1996;75: 27–32.
Wrase J, Reimold M, Puls I, Kienast T, Heinz A. Serotonergic dysfunction: brain imaging and behavioral correlates. Cogn Affect Behav Neurosci 2006;6:53–61.
Wright RC, Ingenito AJ. Blockade of dorsal hippocampal kappa-opioid receptors increases blood pressure in normotensive and isolation-induced hypertensive rats. Neuropeptides 2003;37:127–32.