Brain levels of GABA, glutamate and aspartate in sociable, aggressive and timid mice: An in vivo microdialysis study

Magdaléna Šustková-Fišerová [1], Jaroslava Vávrová [2], Miloslav Kršiak [1]

1. Department of Pharmacology, 3rd Faculty of Medicine, Charles University, Ruská 87, Prague 10, 100 34, Czech Republic.
2. Department of Clinical Biochemistry and Diagnostics, University Hospital, Hradec Králové , Czech Republic.

Correspondence to: Dr. Magdaléna Šustková,
Department of Pharmacology, 3rd Faculty of Medicine, Charles University,
Ruská 87, Prague 10, 100 34, Czech Republic.
PHONE: +420 267102402, E-MAIL: magda.sustkova@volny.cz

Submitted: 2008-10-09 Accepted: 2008-10-12 Published online: 2009-03-10

Key words: **GABA; glutamate; aspartate; medial prefrontal cortex (mPFC); aggression; anxiety; social behavior; isolation; stress; subtypes; mice; microdialysis**

Neuroendocrinol Lett 2009; **30**(1):79–84 **PMID:** 19300401 NEL300109A19

Abstract

OBJECTIVES: Some individually-housed male mice behave aggressively during encounters with strange males, while others are timid or sociable in the same situation. The objective of the present study was to examine concentrations of glutamate, aspartate, and GABA in the brain of aggressive, timid, and sociable mice.

METHODS: Random-bred albino mice were housed individually for three weeks and then classified in three groups (aggressive, timid, and sociable mice) according to their behavior during social interaction with non-aggressive group-housed male mice in a neutral cage. One week after categorization, by means of the social conflict test, levels of glutamate, aspartate, and GABA were measured by in vivo microdialysis of the medial prefrontal cortex (mPFC) of the isolated and group-housed mice.

RESULTS: Sociable mice had almost triple the levels of GABA in their mPFC than aggressive or timid mice. No significant differences in aspartate and glutamate levels were found in these three types of individually-housed mice. Forebrain chemistry of group-housed mice did not differ from that of individually-housed mice with the exception of levels of glutamate and GABA which were significantly lower in group-housed mice than in sociable individually-housed mice.

CONCLUSION: The present results suggest that GABA might play a role in sociable behavior. Results also corroborate other findings indicating that the GABAergic system represents an important molecular and neuronal substrate for the selective attenuation of anxiety and aggression.

ORIGINAL ARTICLE

INTRODUCTION

It is well known that individually-housed male mice can show a wide spectrum of behaviors and postures when introduced individually to a non-aggressive group-housed male mouse in a neutral cage. Under this arrangement (the social conflict test), some individually-housed mice attack their partners (aggressive mice), others show defensive-escape activities, although their partners are completely non-aggressive (timid mice), or they intensively investigate their partners (sociable mice) without exhibiting any agonistic (aggressive or timid) activity. This experimental model has been used extensively in behavioral pharmacology. Drugs showed characteristic behavioral profiles in aggressive, timid, or sociable mice. For example most drugs possessing anxiolytic activity, in man, reduced active escapes or defenses at doses lower than those inhibiting attacks or locomotion (Krsiak, 1975; Krsiak, 1979; Krsiak *et al*., 1981; Krsiak *et al*., 1984). Ethanol increased aggression in aggressive or timid mice but did not evoke aggression in sociable mice (Krsiak, 1976). It is not known why some isolated mice behave aggressively during encounters with strange males, while others are timid or sociable in the same situation. These three types of animals might differ, not only in their behavior and response to drugs, but also in brain chemistry. The objective of the present study was to examine concentrations of glutamate, aspartate, and GABA in the brain of aggressive, timid, and sociable mice with in vivo microdialysis.

MATERIAL AND METHODS

Subjects. Male albino random-bred mice, derived from the ICR strain (Velaz, Prague, Czech Republic), weighing 18–20 g, at the beginning of experimental housing, were used. They were housed individually in self-cleaning cages or in groups of 10. The cages used for individual housing had solid metal walls 13 cm high with wire-mesh floors (8 × 17 cm), placed 3 cm above trays with wood shavings. This wire-mesh floor ensured that the isolated mice were not handled throughout the period of individual housing. The mice kept in groups were housed in large standard plastic cages (26 × 42 × 15 cm) with floors covered with wood shavings. All mice were housed under room lighting (with lights on from 6 a.m. to 6 p.m.), constant humidity (50–60 %) and temperature (22–24 °C). Food and water were available ad libitum.

Experiments were approved by the Expert Committee for Protection of Experimental Animals of the 3rd Faculty of Medicine, Charles University in Prague and were performed in accordance with the Animal Protection Act of the Czech Republic (No. 246/1992 Sb).

Social interaction tests. The standard procedure for the social conflict test, used in previous studies, was employed (Krsiak, 1976; Votava *et al*., 2001). Social interactions always involved one individually-housed mouse and one group-housed mouse. The isolated mouse was allowed 15 min to adapt to the observational cage before the group-housed mouse was introduced; the interaction ended after 4 min. This procedure, which suppresses aggression in group-housed mice and reduces their social behavior, facilitates active social behavior in isolated mice. The observational cages had transparent walls (20 cm high), transparent covers with apertures for air and wood shavings on the floor (20 × 30 cm). The observation cages were cleaned and their floors were covered with fresh wood shavings after each interaction. The observations were performed under moderate room lighting between 8 a.m. and 1 p.m.

The behavior of animals during the interactions was recorded on videotape. The tapes were later analyzed by an observer. This was done with a key-board that was connected to a standard PC and behavioral analysis software (Donát, 1991).

*Measures***.** The frequency, total duration and latency of the number of aggressive, defensive-escape (timid), social and locomotor activities derived from the ethogram of mice (Grant and Mackintosh 1963) and previously described in detail (Kršiak, 1975; Krsiak, 1979; Votava *et al.* 2001) were recorded. *Sociable activities, acts (social investigation):* Social sniff – sniffing the partner's head, body, genitals or tail; climb– the mouse places its forepaws on the partner's back, mostly in the shoulder region, and usually sniffs this area at the same time; follow – following the partner by quiet walking. *Aggressive activities (acts):* Attack – a fierce lunging at the partner often associated with biting; threat – a sideways or an upright stance with head and fore-body movements toward the partner, and trying to bite the partner (offensive sideways or upright posture); tail rattle – rapid vibrations of the tail. *Timid activities (acts):* Defense – the mouse responds to the partner's social behavior by raising forepaws, hunching the back (defensive upright posture) or by some rotation of the body bringing the legs closest to the other animal off the ground (defensive sideways posture); escape – a rapid running or jumping away from the partner; alert posture – a sudden interruption of all movements with eyes and ears being directed toward the partner. *Locomotor activities (acts):* Walk – any walking across the cage which is not apparently related to the partner; rear – the mouse stands on his hind legs and usually sniffs the air or walls at the same time.

The inter-observer reliability of the recorded items was satisfactory as determined by several observers independently scoring the videotaped record of the behavior of 70 mice in interactions lasting 4 minutes each. The correlation ranged from r = 0.83 to 0.97.

Schedule of experiment. After 3 weeks of isolation, social interactions were performed under conditions described above. According to behaviors exhibited during the interaction, the isolated mice were classified into three groups: aggressive, sociable, or timid mice (see below). One week later an in vivo microdialysis

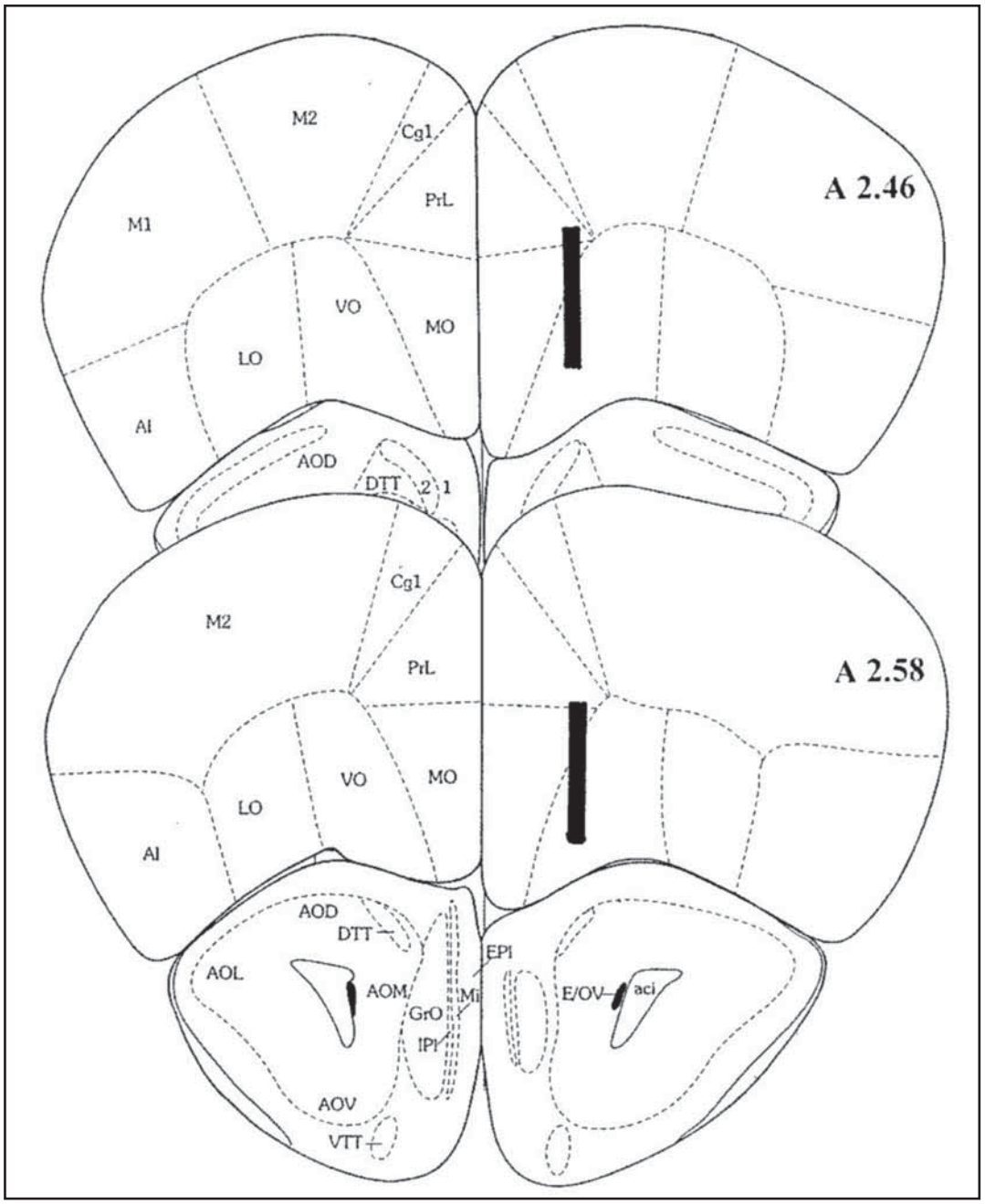

Figure 1. Schematic localization of dialysis probes (dialyzing portion) within the mPFC, as referred to in the atlas of Franklin and Paxinos (1997). On the right of both sections, starting from cranial, is indicated the distance from bregma in mm. The probe is indicated with the bold solid line.

assay was performed on the isolated mice and on the group-housed mice. The medial prefrontal cortex of the mice was dialyzed and basal concentrations of GABA, glutamate and aspartate in the dialysates were subsequently evaluated using CE LIF (see below).

Probe preparation and surgery. Concentric dialysis probes were prepared using a modification of the method described by (Fiserova *et al*., 1999). Each probe was assembled from AN 69 dialysis fibre (sodium sulphate copolymer) (20,000 Da, 310 μm OD, 220 μm ID; Hospal, Dasco, Bologna, Italy), silica fused capillary tubing (ID 75 μm; OD 150 μm), a 30 gauge stainless steel cannula, and polyethylene tubing (ID 0.58 mm; OD 0.96 mm) using Super-Epoxy. The proper dialysis-open area on the dialysis fibre was 1 mm (from the tip). Under Equithesin anesthesia (1% pentobarbital and 4% chloral hydrate), mice were implanted with vertical dialysis probes, using a stereotaxic apparatus. After taking the co-ordinates with the dialysis probe mounted on the stereotaxic holder (medial prefrontal cortex; mPFC: A: +2.5 mm and L: ± 1.3 mm from bregma and V: 5.0 mm from occipital bone) (Franklin and Paxinos 1996) the fiber was slowly lowered into the brain and secured to the skull with dental cement. After completion of the microdialysis experiments the placement of the dialysis probe was verified histologically (Fig. 1). Animals with

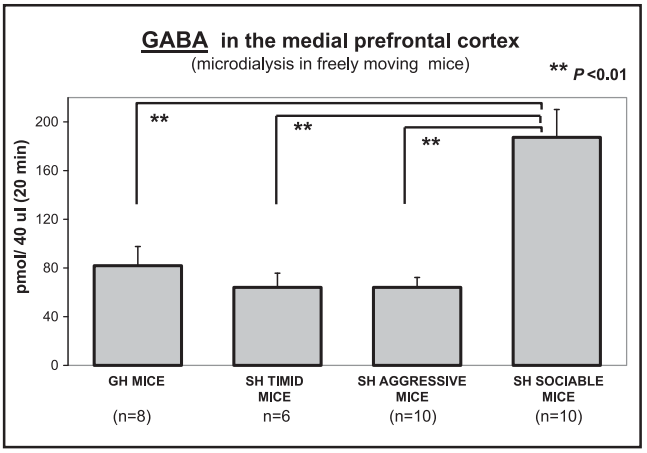

Figure 2. Concentrations of gamma-aminobutyric acid (pmol/40 microl) in dialysates from the medial prefrontal cortex in four categories of mice (means ± SEM). GH = group-housed, SH = singly housed. One-way ANOVA (P<0.001), ** p < 0.01 for subsequent multiple comparisons with the Holm-Sidak method.

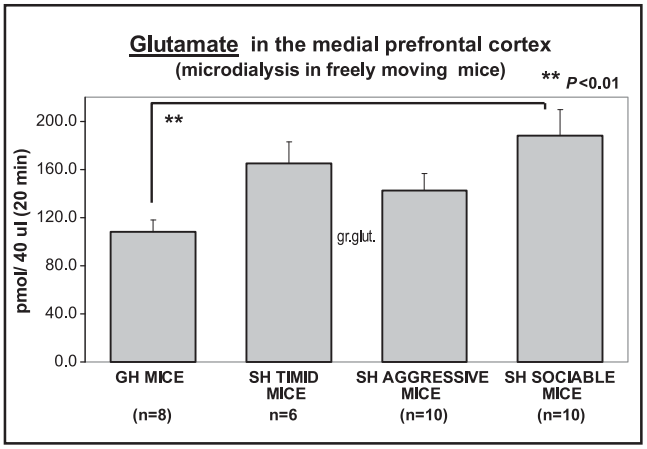

Figure 3. Concentrations of glutamate (pmol/40 microl) in dialysates from the medial prefrontal cortex in four categories of mice (means ± SEM). GH = group-housed, SH = singly housed. One-way ANOVA (P<0.05), ** p < 0.01 for subsequent multiple comparisons with the Holm-Sidak method.

the probe outside the mPFC region were not included in the study results.

Microdialysis and biochemistry. At least 24 h after implantation, the dialysis probe was perfused at a constant rate of 2 µl/min with Ringer's solution. The dialysate was discarded during the first 30 min of dialysis. Subsequently, 40 µl samples were collected, at 20-min intervals in small ice-cooled polyethylene test tubes. Baseline samples were collected during an 80 min equilibration period to allow the neurotransmitter to reach a steady level. Immediately following collection, the samples were frozen on dry ice and lyophilized. GABA contents were quantified with a specific and selective analytical method using capillary electrophoresis with a laser-induced fluorescence detector (CE LIF); GABA concentrations are presented in pmols/ 20 min.

Reagents. All reagents were of analytical grade. Ringer`s solution (147 mM NaCl, 2.2 mM $CaCl_2$ and 4.0 mM KCl adjusted to pH 7.4 with 0.1 N NaOH) was used for dialysis. Gamma-amino-N-butyric acid (GABA; 4-amino-n-butyric acid) was purchased from Sigma-Aldrich Co.

Data analysis. The isolated mice were first classified into one of three groups according to their behavior during social interaction: aggressive mice (exhibiting attacks), timid mice (exhibiting escapes or defensive postures but no attacks), and sociable mice (exhibiting no attacks, defenses or escapes). The procedure used to classify the mice has been previously described (Kršiak 1975). The individual type of behavior (aggressive, timid, or sociable) has been seen to be significantly stable in repeated interactions as shown previously (Kršiak 1975; Donát 1986; Kršiak and Šulcová 1990).

The concentrations of GABA, glutamate, and aspartate in the dialysates from brains of the three categories of isolated mice or group-housed mice were statistically analyzed using a one way analysis of variance followed by the Holman-Sidak method for multiple comparisons.

RESULTS

GABA concentrations

There was a significant difference in the mean values of GABA concentrations in dialysates from the medial prefrontal cortex among individually-housed timid, aggressive, and sociable mice and group-housed mice (F (3, 30) = 14.714, P < 0.001, one way ANOVA). Levels of GABA were markedly and significantly higher in the medial prefrontal cortex of individually-housed sociable mice than in individually-housed aggressive mice, individually-housed timid mice and group-housed mice (t = 5.820, 5.038, and 4.937, respectively, P < 0.01, Holman-Sidak method, Fig. 2). No differences were found in dialysate GABA levels in brains of individually-housed timid mice, individually-housed aggressive mice, and group-housed mice (Fig. 2).

Glutamate concentrations

There was a significant difference in mean values of glutamate concentrations in dialysates from the mPFC in timid, aggressive and sociable individually-housed and group-housed mice (F (3, 30) = 3.441, P < 0.05, one way ANOVA, Fig. 3). Pair-wise multiple comparisons (Holm-Sidak method) revealed only one significant difference; glutamate levels were significantly lower in group-housed mice than in individually-housed sociable mice (t = 3.091, P < 0.01, Fig. 3). There was also a significant difference in the mean values of glutamate concentrations between group-housed mice and all individually-housed mice combined (t = –2.690, df = 16, P = 0.016, t-test).

Aspartate concentrations

No significant differences were found among the mean values of aspartate concentrations in mPFC dialysates of timid, aggressive, and sociable individually-housed mice and group-housed mice (F (3, 30) = 0.585, P = 0.630, one way ANOVA, Fig. 4).

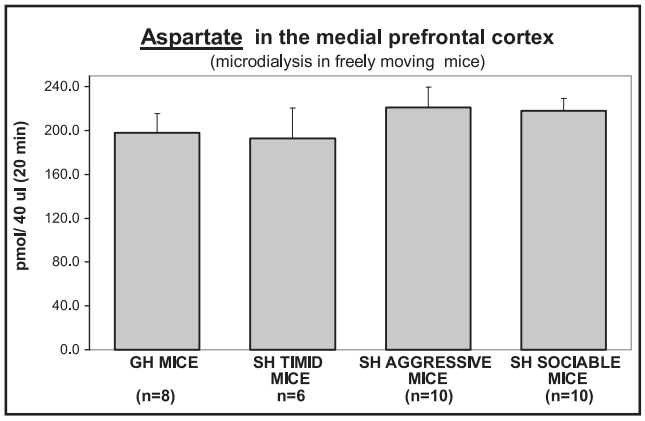

Figure 4. Concentrations of aspartate (pmol/40 microl) in dialysates from the medial prefrontal cortex in four categories of mice (means ± SEM). GH = group-housed, SH = singly housed.

DISCUSSION

The most prominent differences in brain chemistry among aggressive, timid, and sociable individually-housed mice were in levels of GABA: sociable mice had markedly higher levels of GABA in the forebrain than aggressive and timid mice as determined by in vivo microdialysis. On the other hand, no significant differences in aspartate and glutamate levels in the forebrain were found among the three types of mice.

These results corroborate other findings indicating that the GABAergic system represents an important molecular and neuronal substrate for selective attenuation of anxiety and, to some extent, of aggression (Low *et al*., 2000; Miczek *et al*., 1995; Miczek *et al*., 2002). In the present experimental model (i.e. the social conflict test), only drugs stimulating the GABA-receptor complex (benzodiazepines, barbiturates and GABAergic drugs) selectively inhibited active escapes and defenses (i.e. the most conspicuous expressions of timidity) (Krsiak *et al*., 1984). Timid and aggressive mice become less timid or aggressive and more sociable after GABAergic drugs (Krsiak *et al*., 1984; Krsiak & Sulcova, 1990) as if these drugs compensated for a deficiency of GABA in timid and aggressive animals. Undoubtedly, individual differences detected in forebrain chemistry may contribute to the final manifestation of a drug's effect. For example, alcohol, tested in a wide dose range, increased aggressive behavior in aggressive or timid mice, but not in sociable mice (Krsiak, 1976).

All individually-housed mice were randomly selected and subjected to the same form of social isolation, but only some responded to a strange partner with timid or aggressive behavior. Those who did not (sociable mice) had almost triple the levels of GABA in their mPFC. The reasons for this large difference are unclear. Genetic and/or developmental factors might be responsible.

Helmeke *et al*. (2008) found in their work, on the Octodon degus, that early adverse emotional experiences (separation stress) induced long lasting, significantly lower densities of calbindin-D28k-immunoreactive interneurons (including GABAergic neurons) in the medial prefrontal cortex of the degu.

Involvement of the GABA receptor subunit gene in autism risk has been demonstrated (Collins *et al*., 2006). Impaired regulation of the GABAergic system in some individuals with autism has been recently hypothesized (Mercadante *et al*., 2008). Deficits in attachment behavior in mice lacking the mu-opioid receptor gene have been reported (Moles *et al*., 2004). And deficits in social cognition and social behavior have been found in the oxytocin knockout mouse (Winslow & Insel, 2002).

Regarding the differences in brain chemistry between individually-housed and group-housed mice, no significant differences in forebrain glutamate and aspartate levels were found between group-housed and individually-housed aggressive and timid mice. Only individually-housed sociable mice differed from group-housed mice in forebrain chemistry: individually-housed sociable mice had significantly higher levels of GABA and glutamate in the medial prefrontal cortex than group-housed mice.

Literature involving neurochemical and neuroendocrinological consequences of social isolation in mice and rats is quite extensive, often with contradictory results. While some papers offer evidence of the stressful nature of social isolation in mice (e.g. increased corticosterone plasma levels after a prolonged isolation - Frances *et al*., 2000), others have challenged this concept, at least in mice (Brain, 1975; Haller & Halasz, 1999). Indeed, adult male mice usually live, under natural conditions, in large families composed of females and offspring, while adult males tend to be driven away. So under "normal" laboratory monogamous-male-mice-housing conditions, fighting occurs because of intolerance among adult males; dominant males often attack subordinates who then become stressed. Isolated males were, in some respects, more similar to socially dominant males, having heavier testes than subordinated mice (Bartos & Brain, 1993). Social state significantly affected the HPA-axis response in intruder-resident encounters in mice (Pletzer *et al*., 2007). Thus, differences in stress due to group-housing and isolation might contribute to the neurochemical differences between individually-housed sociable mice and group-housed mice.

Acknowledgement
Supported by grant MSM0021620816.

REFERENCES

1 Bartos L, Brain PF (1993). Physiological-responses to social-status and housing conditions in male-mice subject to food competition tests. Bollettino di Zoologia. **60**: 293–296.
2 Brain P (1975). What does individual housing mean to a mouse? Life Sciences. **16**: 187–200.
3 Collins AL, Ma DQ, Whitehead PL, Martin ER, Wright HH, Abramson RK, Hussman JP, Haines JL, Cuccaro ML, Gilbert JR, Pericak-Vance MA (2006). Investigation of autism and GABA receptor subunit genes in multiple ethnic groups. Neurogenetics. **7**: 167–174.
4 Fiserova M, Consolo S, Krsiak M (1999). Chronic morphine induces long-lasting changes in acetylcholine release in rat nucleus accumbens core and shell: an in vivo microdialysis study. Psychopharmacology. **142**: 85–94.
5 Frances H, Graulet AM, Debray M, Coudereau JP, Gueris J, Bourre JM (2000). Morphine-induced sensitization of locomotor activity in mice: effect of social isolation on plasma corticosterone levels. Brain Research. **860**: 136–140.
6 Haller J, Halasz J (1999). Mild social stress abolishes the effects of isolation on anxiety and chlordiazepoxide reactivity. Psychopharmacology. **144**: 311–315.
7 Helmeke C, Ovtscharoff W, Poeggel G, Braun K (2008). Imbalance of immunohistochemically characterized interneuron populations in the adolescent and adult rodent medial prefrontal cortex after repeated exposure to neonatal separation stress. Neuroscience. **152**: 18–28.
8 Krsiak M (1975). Timid singly-housed mice – their value in prediction of psychotropic activity of drugs. British Journal of Pharmacology. **55**: 141–150.
9 Krsiak M (1976). Effect of ethanol on aggression and timidity in mice. Psychopharmacology. **51**: 75–80.
10 Krsiak M (1979). Effects of drugs on behavior of aggressive mice. British Journal of Pharmacology. **65**: 525–533.
11 Krsiak M, Sulcova A (1990). Differential-effects of 6 structurally related benzodiazepines on some ethological measures of timidity, aggression and locomotion in mice. Psychopharmacology. **101**: 396–402.
12 Krsiak M, Sulcova A, Donat P, Tomasikova Z, Dlohozkova N, Kosar E, Masek K (1984). Can social and agonistic interactions be used to detect anxiolytic activity of drugs? In: Miczek KA, Kruk MR, Olivier B, editors Ethopharmacological Aggression Research. New York: Alan R. Liss, Inc.
13 Krsiak M, Sulcova A, Tomasikova Z, Dlohozkova N, Kosar E, Masek K (1981). Drug effects on attack, defense and escape in mice. Pharmacology Biochemistry and Behavior. **14**: 47–52.
14 Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, Rudolph U (2000). Molecular and neuronal substrate for the selective attenuation of anxiety. Science. **290**: 131–134.
15 Mercadante MT, Cysneiros RM, Schwartzman JS, Arida RM, Cavalheiro EA, Scorza FA (2008). Neurogenesis in the amygdala: A new etiologic hypothesis of autism? Medical Hypotheses. **70**: 352–357.
16 Miczek KA, Fish EW, De Bold JF, de Almeida RMM (2002). Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and gamma-aminobutyric acid systems. Psychopharmacology. **163**: 434–458.
17 Miczek KA, Weerts EM, Vivian JA, Barros HM (1995). Aggression, anxiety and vocalizations in animals – Gaba(A) and 5-ht anxiolytics. Psychopharmacology. **121**: 38–56.
18 Moles A, Kieffer BL, D'Amato FR (2004). Deficit in attachment behavior in mice lacking the mu-opioid receptor gene. Science. **304**: 1983–1986.
19 Pletzer B, Klimesch W, Oberascher-Holzinger K, Kerschbaum H (2007). Corticosterone response in a resident-intruder-paradigm depends on social state and coping style in adolescent male Balb-C mice. Neuroendocrinology Letters. **28**: 585–590.
20 Votava M, Krsiak M, Podhorna J, Miczek KA (2001). Alprazolam withdrawal and tolerance measured in the social conflict test in mice. Psychopharmacology. **157**: 123–130.
21 Winslow JT, Insel TR (2002). The social deficits of the oxytocin knockout mouse. Neuropeptides. **36**: 221–229.