Pharmacology, Biochemistry and Behavior 152 (2017) 90–96

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Head shaking in the forced swim test: A robust but unexplored sex difference

Nikolaos Kokras [a,b], Alexia Polissidis [a,c], Katerina Antoniou [d], Christina Dalla [a,*]

[a] Department of Pharmacology, Medical School, University of Athens, Greece
[b] First Department of Psychiatry, Eginition Hospital, Medical School, University of Athens, Greece
[c] Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[d] Department of Pharmacology, Medical School, University of Ioannina, Greece

ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form 4 May 2016
Accepted 12 May 2016
Available online 13 May 2016

Keywords:
FST
Head swinging
Head twitching
Antidepressant
Depression
Sex differences

ABSTRACT

Preclinical psychopharmacology research needs novel behavioral indices and improved animal models for both sexes. The forced swim test (FST) is the most popular test for screening antidepressant potential. Sex differences in FST behaviors, such as immobility and swimming, are not consistent among laboratories. Reliable indices, sensitive to sex differences, are required. We identified a robust sex difference in the frequency of headshakes during the standard two session FST, with male rats exhibiting higher number of head shakes than females. Furthermore, we explored whether strain, ageing, sex- and stress-hormone levels influence this sex difference. Experiments in middle-aged and senescent Wistar rats, as well as in gonadectomized and adrenalectomized with corticosterone replacement young adult males and females, revealed that sex differences in headshakes during FST are not influenced by age or corticosterone, but are abolished following castration of male rats. Interestingly, headshake frequency correlated positively with testosterone, but not corticosterone levels. Finally, testing of Flinders Sensitive Line (FSL) and Sprague-Dawley (SD) rats in a single 5 min FST session revealed that headshake frequency is sensitive to antidepressant treatment with female rats exhibiting opposite responses to treatment than male FSL rats. Mirtazapine, a 5-HT2 antagonist, enhanced headshakes in females and decreased them in male FSL rats. Based on current data and the available literature, sex differences in headshake frequency should be linked to analogous sex differences in serotonin receptors. Headshake frequency during the FST is an additional valuable behavioral index, sensitive to sex differences, gonadal hormones and antidepressants modulating serotonin receptors.

1. Introduction

Lately, there is a growing concern regarding the credibility and reproducibility of preclinical studies in psychopharmacology (Steckler et al., 2015). Among many others, one factor that often influences the outcome of experimental studies is the sex of the animals (Dalla et al., 2005; Dalla et al., 2008a; Dalla et al., 2010, 2011; Kokras and Dalla, 2014). Hence the NIH recently issued a new guideline asking for a justification of the choice of the sex of the animals in all studies (Clayton and Collins, 2014). Especially in the case of depression, which is twice more frequent in women than in men (Marcus et al., 2005; Sloan and Kornstein, 2003), the use of both sexes in preclinical studies is now considered to be the best practice (Kokras et al., 2015).

Furthermore, it is known that we need appropriate and improved behavioral indices in order to identify psychotropic actions of new molecules in both sexes. The animal test that is most frequently used to detect antidepressant potential is the forced swim test (FST) (Cryan et al., 2005; Porsolt et al., 1979a; Porsolt et al., 1979b). Recently, we have thoroughly discussed sex differences in this test and we have identified large differences in procedures used by different laboratories (Kokras et al., 2015). As a result, sex differences in immobility, swimming and climbing behaviors are not consistent among different laboratories. However, through our studies we have identified a behavioral index in the rat FST that is easy to score and standardize, and it is characterized by marked sex differences. This behavior, which consists of an abrupt movement of the head that the rat is doing when its head is outside the water (Lino-de-Oliveira et al., 2005; Pare, 1989), was originally referred as "head shakes" in the FST, but the terms "head swinging" and "head twitching" are also used in the literature. At the seminal study of Barros and Ferigolo (1998), female rats had lower numbers of headshakes than males and headshakes were further decreased by imipramine treatment in both sexes (Barros and Ferigolo, 1998). However, in later studies this behavior was no longer monitored by most researchers (Cryan et al., 2005). In our FST studies we consistently find

* Corresponding author at: Department of Pharmacology, Medical School, University of Athens, Mikras Asias 75, Athens 11527, Greece.
E-mail address: cdalla@med.uoa.gr (C. Dalla).

http://dx.doi.org/10.1016/j.pbb.2016.05.007
0091-3057/

that male rats exhibit more headshakes than females (Drossopoulou et al., 2004; Kokras et al., 2009a; Kokras et al., 2015; Kokras et al., 2014). From those studies there are indications that antidepressant treatment and variations in levels of sex hormones may influence headshake frequency in the FST, however sex differences in this behavior still remain largely unexplored.

Therefore, in this study we planned a series of experiments in order to confirm our hypothesis that head shaking in the FST presents a significant sex difference, with male rats shaking their head more frequently than females. Additionally, head shaking has not been previously studied in relation to factors, such as gonadal and stress hormones, ageing and strain differences, which are known to influence immobility, climbing and swimming during FST (Bogdanova et al., 2013; Estrada-Camarena et al., 2006; Gomez et al., 2014; Martinez-Mota et al., 2008; Olivares-Nazario et al., 2016; Recamier-Carballo et al., 2012). Based on previous FST data from our group (Drossopoulou et al., 2004; Kokras et al., 2009a; Kokras et al., 2015; Kokras et al., 2014), we hypothesized that sex differences in head shaking frequency would not depend on corticosterone or age of the rats. Instead, we hypothesized that head shaking would depend on 5-HT receptors and gonadal hormones, as they are known to modulate 5-HT receptors. In this context we further hypothesized that 5-HT receptor supersensitive animals, like the Flinders Sensitive Line (FSL) of rats would present significantly less head shakes and that a 5-HT_{2A} receptor antagonist, namely the antidepressant mirtazapine, would modulate head shaking frequency in these animals in a sex-dependent manner.

2. Material and methods

2.1. Animals

All animals were group housed at the Department of Pharmacology, Medical School, University of Athens, unless otherwise noted, under standard laboratory conditions in cages measuring L570 × W380 × H200 mm under controlled light/dark (12 h cycle, lights on at 08:00 a.m.), temperature (22 ± 2 °C) and humidity (40–60%) conditions with free access to food pellets (2% fat, 16.5% protein) and tap water, unless otherwise noted. All efforts were made to minimize animal suffering and to reduce the number of animals used. Experiments were carried out in accordance with the directives 86/609/EEC and 2010/63/EU (Council of the European Union, 1986, 2010).

2.2. Experiment 1: Effect of gonadectomy

Male and normal cycling female adult Wistar rats (each group n = 12) weighing 350 ± 13 and 250 ± 8 g (mean ± SEM) respectively and aged 3 months, were used. Six females received a bilateral ovariectomy, six males a castration (orchidectomy) and the remaining animals a sham operation (consisting of a similar incision and suturing) under anesthesia with 100 mg/kg ketamine, 10 mg/kg xylazine and 0.5 mg/kg atropine intraperitoneally (i.p.) as described in Dalla et al. (2008b). After surgery, rats were kept warm and under observation until recovery from anesthesia. After surgery rats were provided or.syr. acetaminophen 24 mg/ml, at a 100 mg/kg dose, administered orally. All animals were left undisturbed to recover in their cages for 3 weeks before FST exposure. Immediately after FST, all rats (controls and FST) were killed by rapid decapitation and trunk blood was used for serum extraction, as previously performed in our laboratory (Kokras et al., 2015; Kokras et al., 2014; Mikail et al., 2012). Blood samples were processed to recover serum (centrifugation at 4000g, 30 min, 4 °C); and the serum samples were stored at −20 °C before analysis for corticosterone and testosterone. Corticosterone was assayed by a standard RIA (MP Biomedicals, Costa Mesa, CA), as previously described (Kokras et al., 2012). The inter- and intra-assay coefficients of variation were both 8%. Testosterone assays were also performed in serum using commercially available RIA kits (Siemens Coat-A-Count for Total Testosterone), as before (Kokras et al., 2015). The detection limit for testosterone was 0.4 ng/ml.

2.3. Experiment 2: Effect of adrenalectomy and stable corticosterone replacement

Male (n = 23) and female Wistar rats (n = 25), weighing 300 ± 40 g and 210 ± 20 g, respectively, and aged 3 months at the beginning of the experiment, were used. Thirteen male and thirteen female rats were subjected to an adrenalectomy and the remaining rats to a sham operation under anesthesia with 100 mg/kg ketamine, 10 mg/kg xylazine and 0.5 mg/kg atropine intraperitoneally, as described in more detail in Kokras et al. (2012). After surgery, rats were kept warm and under observation until recovery from anesthesia and were provided or.syr. acetaminophen 24 mg/ml, at a 100 mg/kg dose, administered orally. Immediately after surgery and continuously throughout the experiment all rats were offered ad libitum a drinking solution consisting of 0.9% NaCl and 0.2% ethanol dissolved in tap water. ADXR rats were also receiving in their drinking solution corticosterone (C2505, Sigma Aldrich, St. Louis, MO) to a final concentration of 25 μg/ml. This method of corticosterone replacement has been shown to mimic the circadian secretion of endogenous corticosterone and this particular dose was suggested to produce basal levels of corticosterone and ACTH. Rats were allowed to recover from surgery and adjust to the replacement treatment for 28 days before any behavioral testing was performed. Estrous cycle phases were monitored by vaginal smears as described elsewhere (Becker et al., 2005; Dalla et al., 2008b; Dalla et al., 2009). Corticosterone was assayed in blood samples as explained in Experiment 1.

2.4. Experiment 3: effect of ageing

Male and female Wistar rats aged 9 months (n = 6 for each sex) and male and female Wistar rats aged 18 months old (n = 7 for each sex) were used in this study. Following estrous cycle monitoring for 28 days, it was confirmed that 9 months old female rats had a normal estrous cycle of 4–5 days. Selected 18 months old female Wistar rats were deemed to be in senescence when repeated estrous cycle monitoring determined that they were in persistent vaginal cornification (constant estrous) or (less often) in persistent anestrous, in accordance with previous reports (LeFevre and McClintock, 1988, 1991; vom Saal and Finch, 1988; Westwood, 2008).

2.5. Experiment 4: effect of mirtazapine treatment and rat strain

Adult male (n = 19) and female (n = 20) Flinders Sensitive Line (FSL) rats, weighing 250 ± 50 g and 220 ± 30 g, respectively, and aged 2–3 months at the beginning of the experiment, were used. Male (n = 19) and female (n = 20) Sprague-Dawley (SD) rats of similar age, weighing 330 ± 50 g and 250 ± 30 g, respectively, were also used. Some FSL and control SD rats were injected i.p. once daily for 14 days with mirtazapine 10 mg/kg (FSL Males: 9, FSL Females: 10, SD Males: 9, SD Females: 10) and the remaining rats were injected with the same volume of vehicle (sterile water). This dose of mirtazapine has been used previously and was found effective (Gerrits et al., 2006; Kokras et al., 2009b; Owen and Whitton, 2006). Unlike SSRI/SNRIs which act primarily through inhibition of the monoamine transporters, mirtazapine is a weak 5-HT_{1A} and a potent 5-$HT2_{A/C}$ and 5-HT_3 antagonist (Anttila and Leinonen, 2001; Kent, 2000). Given the hypothesized relation of head shaking with 5-HT receptors, mirtazapine was therefore chosen as the most appropriate antidepressant to explore this relation. Additionally, the FSL rat was chosen as a model of depression known for 5-HT receptor supersensitivity (Overstreet et al., 2005; Overstreet and Wegener, 2013).