ELSEVIER

Pharmacology, Biochemistry and Behavior 73 (2002) 703–712

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Fluoxetine-induced increases in open-field habituation in the olfactory bulbectomized rat depend on test aversiveness but not on anxiety

Adam Mar[a], Emma Spreekmeester[a], Joseph Rochford[a,b,*]

[a]*Department of Neurology and Neurosurgery, McGill University, Douglas Hospital Research Centre, Verdun, Quebec, Canada H4H 1R3*
[b]*Department of Psychiatry, McGill University, Douglas Hospital Research Centre, Verdun, Quebec, Canada H4H 1R3*

Received 10 December 2001; received in revised form 18 April 2002; accepted 16 May 2002

Abstract

Little is known regarding the functional processes underlying the treatment efficacy of antidepressant drugs. Given the close association between stress, anxiety and depression, distinguishing the common and disparate features of these processes may contribute to our current understanding. Using the olfactory bulbectomized (OBX) rat, an animal model sensitive to a variety of antidepressant drugs, this study examined the effects of chronic fluoxetine administration on open-field behavior under different conditions of stressfulness (luminance) and compared the fluoxetine effects to those evoked by the anxiolytic lorazepam. Sham-operated and OBX rats received 21 daily injections of fluoxetine (10 mg/kg), one or seven injections of lorazepam (0.1 and 0.5 mg/kg) or vehicle prior to testing in the open field or plus maze. Time series data were collected and fit with exponential regression models to estimate behavioral reactivity, habituation and residual rate of responding. Relative to sham controls, OBX rats displayed increased locomotor activity in the high luminance open field but showed decreased activity in the lower luminance open field. Time series analysis revealed that while sham animals showed increased habituation in the high compared to lower luminance open field, OBX rats did not significantly modify their responding between the two conditions. Chronic fluoxetine treatment invoked rectifying effects in OBX animals only in the high luminance open field by increasing the rate of habituation. Both acute and subchronic administration of lorazepam also reduced OBX hyperactivity but did so only by decreasing the residual rate of responding. As expected, lorazepam administration significantly increased the ratio of open-to-total arm activity in the elevated plus maze. These findings suggest that OBX responding in the open field may be maladaptive, reflecting an inability to modify behavior appropriately in certain environmental contexts. Chronic antidepressant treatment enhances habituation of OBX animals only under more stressful or aversive conditions and appears to do so in a manner temporally distinct from anxiolytic treatment.

Keywords: Antidepressants; Fluoxetine; Stress; Anxiety; Anxiolytics; Lorazepam; Open field; Random effects models; Olfactory bulbectomized rat

1. Introduction

Bilateral olfactory bulbectomy (OBX) in the rat results in a characteristic behavioral phenotype including disrupted circadian patterns in sleep (Araki et al., 1980; Sakurada et al., 1976), feeding (La Rue and Le Magneu, 1972; Meguid et al., 1993) and activity (Giardina and Radek, 1991; Marks et al., 1971) along with increased irritability, aggression and locomotion when confronted with certain novel stimuli (Jesberger and Richardson, 1986; Van Riezen and Leonard, 1990). As many of the OBX-induced behavioral changes are not seen following destruction of the nasal mucosa via zinc sulfate ($ZnSO_4$) irrigation (Alberts and Friedman, 1972; Edwards, 1974; Sieck and Baumbach, 1974), these changes are believed to result from neurochemical dysfunction in brainstem, limbic and/or cortical areas (Hirsch, 1980; Leonard and Tuite, 1981). A particularly attractive feature of the OBX model is the attenuation of most of these behavioral and neurochemical alterations following chronic, but not acute, antidepressant treatment (Cairncross et al., 1978, 1979a; Jesberger and Richardson, 1986; Kelly et al., 1997; Van Riezen et al., 1976).

One of the more widely used behavioral indicators of antidepressant activity in the OBX model is a reduction of the hyperactivity typically exhibited by OBX animals in the

* Corresponding author. Douglas Hospital Research Centre, Lehmann Pavilion, 6875 LaSalle Boulevard, Verdun, Quebec, Canada H4H 1R3. Tel.: +1-514-761-6131x4931; fax: +1-514-762-3048.
E-mail address: rocjos@douglas.mcgill.ca (J. Rochford).

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)00881-X

open-field test. In rodents, the open field is generally considered to be an ethological test measuring the behavioral responses to a natural conflict between exploration of and aversion to open, bright areas. Accordingly, the hyperactivity of OBX rats is believed to reflect an increased responsiveness to the aversive nature of the task (Cairncross et al., 1979b; Kelly et al., 1997; McNish and Davis, 1997; Primeaux and Holmes, 1999; Van Riezen and Leonard, 1990). Surprisingly, little is known about the nature of the functional processes underlying the increased responsiveness of OBX animals or the attenuation of this responsiveness by antidepressant drugs.

Recently, through temporal analysis of open-field activity, we distinguished two components underlying OBX hyperactivity: a higher initial reactivity upon introduction to the open field and the lack of a compensatory increase in habituation to the test (Mar et al., 2000). We further showed that antidepressants appear to rectify the OBX hyperactivity by selectively increasing the rate of habituation. To better interpret these findings, it remains to be demonstrated whether and to what extent these antidepressant-induced increases in behavioral habituation might depend on factors such as test novelty, aversiveness or anxiety as well as possible sedative properties of these drugs.

There is evidence to suggest that the hyperactivity of OBX animals may be sensitive to variations in the testing apparatus and conditions (Kelly et al., 1997; Primeaux and Holmes, 1999). For instance, several studies measuring the activity of OBX animals upon exposure to novel activity boxes, running wheels or mazes have reported either hypoactivity (Cain and Paxinos, 1974; Sieck, 1972) or no significant differences when compared to sham-operated controls (Van Riezen and Leonard, 1990). Moreover, reduced locomotion in OBX animals relative to controls has been observed in a more dimly lit, square open field (Stockert et al., 1988). Though there is some debate as to whether direct illumination or the reflective properties of the apparatus are the critical determinants of OBX hyperactivity, it is clear that either of these variables can be associated with increases in measures of stress and/or anxiety (Ader, 1969; Dalley et al., 1996; Igarashi and Takeshita, 1995; Seliger, 1977; Williams, 1971).

In light of these data, the present experiments were conducted to further our understanding of the behavioral systems underlying the effects of chronic antidepressant administration in OBX animals in the open field. Firstly, we sought to discern whether the observed increases in the habituation rates of OBX animals due to chronic antidepressant administration were dependent upon the stressfulness of the testing conditions. We tested this by evaluating the effect of chronic fluoxetine on behavioral habituation in open fields having very different luminance properties. If antidepressants do increase habituation only in stressful or aversive conditions, then one would expect smaller increases in habituation in a low compared to a high luminance open field. Our second objective was to determine whether the effects of chronic antidepressants might be attributed to their known anxiolytic properties. To test this, we compared the effects of acute and subchronic benzodiazepine (lorazepam) treatment on reactivity and habituation measures in the standard, high luminance open field to those of chronic fluoxetine. As a positive control for the anxiolytic properties of lorazepam, animals were also tested in the elevated plus maze, a validated screen for anxiolytic effects in the rat (Pellow et al., 1985).

2. Materials and methods

2.1. Animals and housing conditions

Male Sprague–Dawley rats ($n = 145$) (Charles River, St Constant, Quebec), weighing 325–375 g upon arrival, were housed two per polycarbonate cage measuring $60 \times 30 \times 25$ cm. They were maintained on a 12:12 h light/dark cycle (lights on 08:00–20:00 h) in a room of ambient temperature (19–22 °C). Rats were allowed free access to food and water for the duration of the experiment and were given 7–9 days to acclimate to laboratory conditions before surgeries were performed. All surgical procedures and behavioral testing procedure complied with the guidelines of the Canadian Council on Animal Care and were approved by the McGill University Animal Care Committee.

2.2. Surgical procedure

Bilateral OBX was performed with rats anesthetized under xylazine (5 mg/kg im) in combination with ketamine hydrochloride (50 mg/kg im) 10 min after an injection of acepromazine (0.5 mg/kg im). A 2 mm hole was drilled 8 mm anterior to bregma on the midline and the olfactory bulbs were severed using fine forceps and removed by aspiration. The cagemate of each OBX rat was sham operated, undergoing identical anesthetic and drilling procedures as OBX animals, but their bulbs were left intact. Animals were given 8–10 days to recover following surgery before chronic antidepressant administration.

Anosmia in OBX animals was assessed after all other behavioral testing was completed by examining responses to the presentation of a novel odor. A small cloth soaked in vanilla or almond extract was presented on the top back corner of the animals' home cage while the experimenter observed from the front. Rats were considered anosmic if they did not meet at least two of the following criteria within a the 90 s test session: (1) more than one approach to the odor source, (2) more than 10 s sniffing the odor source and (3) a qualitative increase in sniff rate at the odor source. Two testing sessions were performed to help control for possible visual cues or activational effects from an animal's cagemate.

2.3. Drugs

Fluoxetine hydrochloride was purchased from Laboratoires Denis Giroux (Saint-Pierre, Quebec). Sterile, injectable Lorazepam (4.0 mg in 0.18 ml polyethylene glycol 400 in propylene glycol with 2.0% benzyl alcohol as preservative) was purchased from Wyeth-Ayerst (Montreal, Quebec).

2.4. Drug treatment

All drugs were injected intraperitoneally in a volume of 1 ml/kg between 10:00 and 12:00 h. Fluoxetine and lorazepam were dissolved using deionized water as vehicle and were prepared fresh each morning. For chronic antidepressant administration, sham-operated and OBX animals were given either vehicle or fluoxetine (10/mg/kg) once daily for 21 days. A 48 h washout period was observed after the last injection to minimize possible acute drug effects (Caccia et al., 1990; Gardier et al., 1994). For acute anxiolytic drug treatment, animals (which had received 21 days of vehicle injections) were given either vehicle or lorazepam (0.1 or 0.5 mg/kg) 30 min prior to behavioral testing. As a single injection of lorazepam is known to induce some sedative effects (File, 1981, 1984), a group was included in which animals were injected with vehicle for 17 days, preceding six consecutive daily injections with 0.1 mg/kg lorazepam, including the day of (30 min prior to) behavioral testing.

2.5. Behavioral testing

In Experiment 1, different groups of rats were tested either in the high luminance or in the lower luminance open field. In Experiment 2, rats were tested in both the high luminance open field and the elevated plus maze in a counterbalanced design over 2 consecutive days. All testing was conducted between 10:00 and 16:00 h and each test apparatus was cleaned thoroughly between animals.

2.5.1. Open-field test

The open-field apparatus consisted of an $85 \times 85 \times 70$ cm wooden box painted flat black. This apparatus, in conjunction with normal room lighting (provided by two 40 W fluorescent tubes in an overhead fixture), comprised the lower luminance condition (150–200 lx). For the high luminance condition, the floor and inner walls were lined with aluminum foil and lighting was provided by a 100 W bulb hanging 100 cm directly over the center of the open field (1500–2000 lx). For scoring, the field was divided into 25 squares measuring 17 cm^2. A square entry was assigned whenever a rat placed both front paws into a new square. At the start of each test, a rat, taken pseudorandomly between groups, was placed into the center square of the open field. Each 5 min trial was videotaped by an overhead camera and was scored later by two independent raters using a home-made computer program, which enabled instantaneous encoding of behavior type (square entry, rearing and grooming), square position and time, so that behavioral patterns could be reconstructed and analyzed.

2.5.2. Elevated plus maze

The elevated plus maze was made of wood covered with white enamel and had four arms 50 cm long and 10 cm wide at a height 53 cm above the floor. The two enclosed arms had 40 cm high walls. The maze was illuminated using diffuse overhead fluorescent lighting similar to the lower luminance open field ($\sim$50 lx for closed arms and 150–200 lx for open arms). At the start of each test, animals were placed in the center of the maze facing an open arm. Trials lasted 5 min and were videotaped and encoded as described for the open field (i.e., in addition to open and closed arm entries, each arm was divided into four 12.5×10 cm squares to provide a more sensitive index of activity in each arm. As square size was similar to that in the open field, it also enabled comparisons between activity levels in the two tasks).

2.6. Statistical methods

To analyze measures cumulated over the entire duration of each test, an ANOVA with independent samples was used with surgery (sham and OBX) and drug treatment (vehicle, fluoxetine or vehicle, lorazepam) as factors. Significant interactions were decomposed using simple main effect F tests. When required, pairwise contrasts of main effects or simple main effects were conducted using Dunnett's test by using the appropriate vehicle-treated group as controls.

Time series data were extracted by taking totals for each measure at 20 s intervals. Curves for each group of animals were constructed and fit with a random effects exponential regression model (implemented using the NLINMIX macro within the SAS statistical system v6.12) as indicated below:

$$\text{Behavior} = A \times \exp^{(B \times \text{time})} + C$$

The A-parameter (y-intercept) was operationally defined as reflecting the initial behavioral reactivity of the animal while the B-parameter (nonlinear slope estimate) was defined as the rate of habituation. Various combinations of random and fixed coefficients were examined for these parameters, and due to considerable variability between individual animals within a group, both parameters A and B were included as random effects. The C-parameter reflected any residual steady rates of responding that occurred towards the end of the test session (i.e., activity level observed following habituation) and was included as a random effect only when likelihood ratio testing (see below) confirmed that it provided a significant improvement in fit.

To assess goodness of fit, each regression model was also estimated using a least-squares approach and calculations of R^2 values indicated that all models accounted for at least

70% of the variance on the averaged data from each group of animals in each test. Linear and quadratic functions were also calculated using least squares for each data set but in each case yielded lower R^2 values than the respective exponential function.

Statistical comparisons of the regression parameters (A, B or C) between any two groups were made using a likelihood ratio test. Briefly, the minimum objective function (-2 log-likelihood) of fits in which the parameters of the curves were allowed to vary (full model) was compared with the objective functions of fits in which one of the parameters was constrained to be equal (reduced models). The difference between these objective functions has a χ^2 distribution with the difference in the number of parameters in the full and reduced models as the degrees of freedom. The accepted level of significance was a difference in objective functions associated with a P-value of $<.05$. In addition, all statistical comparisons were verified by evaluating the difference between the parameters against their pooled asymptotic standard error and assessed for significance employing a Bonferroni t correction to control for possible inflation of the α level.

3. Results

Of the 145 rats, 5 died during or in the days following the surgical procedures. After all behavioral testing, histology confirmed the extent of OBXs. Two animals showed considerable damage to frontal cortical areas and their data were excluded from further analyses. Few animals showed a small amount of residual tissue beyond the olfactory tubercle. However, their weights and behavioral data remained consistent with those of other OBX animals. The extent of olfactory impairment was evaluated using observational criteria of olfactory behavior (see Materials and methods) upon two separate presentations of a novel odor (vanilla or almond extract) over the home cage. None of the OBX rats met the criteria over both presentations while only five sham animals failed to meet them, functionally corroborating, at least grossly, the histological results. As, histologically, these five sham animals appeared normal and the behavioral results did not differ greatly in their presence or absence (not shown), their data were included in all analyses.

3.1. Experiment 1: effects of OBX and chronic fluoxetine treatment in open fields with different luminance

3.1.1. High luminance open field

Fig. 1A presents the total locomotor activity scores of vehicle-treated and fluoxetine-treated sham and OBX rats exposed to a 5 min session in a novel, high luminance open field. A two-factor (surgery and drug) independent-groups ANOVA revealed a significant Surgery $\times$ Drug interaction [$F(1,31)=4.50$, $P<.05$]. Simple effects tests revealed that vehicle-treated OBX rats exhibited increased locomotion relative to vehicle-treated sham controls. Fluoxetine appeared to rectify this hyperactivity, as no differences were observed between fluoxetine-treated sham and fluoxetine-treated OBX animals.

Fig. 1B shows the time course of the mean locomotor activity for vehicle-treated sham and OBX animals as well as for fluoxetine-treated OBX animals, with activity scores totaled at 20 s intervals. The fitted exponential random effects models, estimated for each group, are also displayed. Qualitatively, vehicle-treated OBX animals appeared to be initially more responsive than sham controls but appeared to habituate at a parallel rate. Furthermore, fluoxetine treatment seemed to rectify OBX hyperactivity not by lowering initial reactivity but by increasing habituation rate (i.e., a greater decrement in activity over the first 20–60 s) to reach activity levels comparable to that of shams. We statistically assessed these observations by conducting likelihood ratio tests on the parameters estimated from the fitted exponential models (Table 1). Compared to vehicle-treated sham animals, both vehicle-treated and fluoxetine-treated OBX animals displayed a significantly increased [$\chi^2(1)=10.6$, $P<.01$] initial reactivity (A-value) upon introduction into the novel, high luminance open field. Fluoxetine-treated OBX animals also showed a significantly increased [$\chi^2(1)=6.0$, $P<.05$] habituation rate (B-value) relative to vehicle-treated OBX animals.

3.1.2. Lower luminance open field

Fig. 1C presents the number of square crosses of vehicle-treated and fluoxetine-treated sham and OBX rats exposed to a 5 min session in a novel, lower luminance open field. A two-factor ANOVA revealed only a significant main effect of surgery [$F(1,31)=9.97$, $P<.01$], in which, in contrast to the high luminance open field, OBX animals showed decreased locomotor activity as compared to sham controls.

Fig. 1D presents the time course of the mean locomotor activity for vehicle-treated sham and OBX animals as well as for fluoxetine-treated OBX animals, with activity scores totaled at 20 s intervals. The fitted exponential models for each group are also displayed. The estimated parameters (initial reactivity and habituation) are shown in Table 1. Note that, in contrast to the high luminance open field, initial locomotor responses in vehicle-treated and fluoxetine-treated sham animals approached those of OBX and no significant differences were observed [$\chi^2(1)=0.2$ and 1.1, P's $>.05$]. Furthermore, both vehicle-treated and fluoxetine-treated sham animals habituated at a slower rate [$\chi^2(1)=3.6$ and 7.6, $P=.058$ and $<.01$] than their respective OBX animals. Finally, in the lower luminance open field, fluoxetine did not alter any of the parameters relative to those of vehicle-treated animals. For both the high and the lower luminance open fields in this experiment, adding a parameter to account for residual rates of responding (C-value) did not significantly improve the fits and was therefore not included.

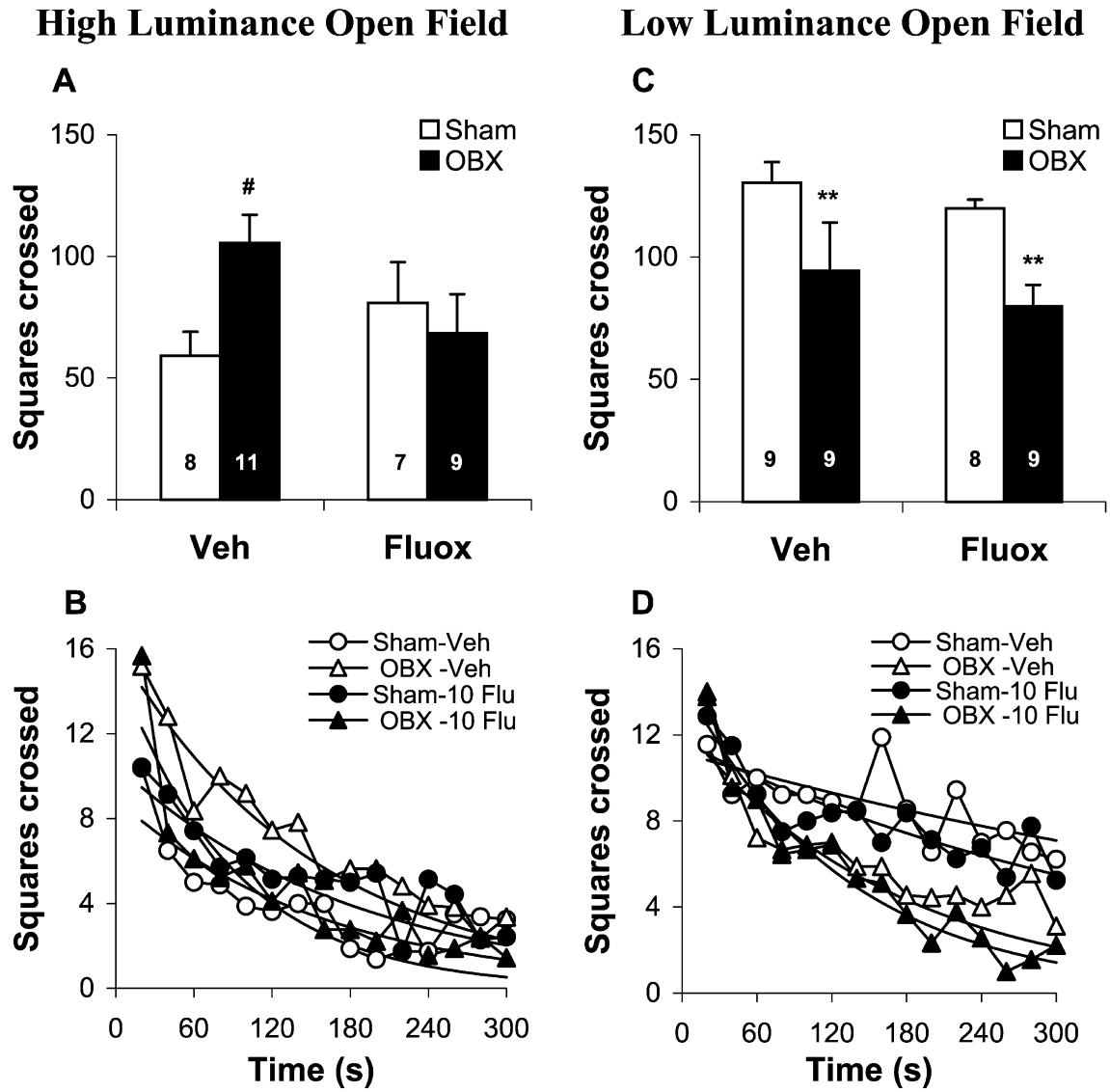

Fig 1. (A) Mean ± S.E.M. number of squares crossed over 5 min in a high luminance open field for sham and OBX rats treated for 21 days with either vehicle or fluoxetine (10 mg/kg). The number of animals per group is shown at the base of each column. (B) Time course of mean squares crossed in a high luminance open field for vehicle-treated and fluoxetine-treated sham and OBX animals, with crossings summed over consecutive 20-s periods. Smooth lines represent fitted exponential models Behavior = $A \times \exp^{(B \times \text{time})}$ with parameters A and B estimated as random effects. (C) Mean ± S.E.M. number of squares crossed over 5 min in a lower luminance open field for sham and OBX rats treated with either chronic vehicle or fluoxetine. (D) Time course of mean squares crossed in a lower luminance open field for vehicle-treated and fluoxetine-treated sham and OBX animals. ** Significant main effect at $P < .01$ relative to sham controls. [#]Significant interaction and simple effect at $P < .05$ relative to vehicle-treated sham controls.

3.1.3. Comparisons between open fields

To compare between the two open-field conditions, we performed a three-factor ANOVA, including luminance as one of the factors. There was a significant Luminance × Surgery interaction [$F(1,62) = 8.98$, $P < .01$], in which sham animals showed significantly different activity scores between the two luminance conditions. Shams were significantly less active in the high luminance open field (Fig. 1A vs. Fig. 1C). The activity of OBX animals was comparable across open-field conditions.

Statistical examination of the time courses of locomotor responses across both open-field conditions revealed that initial reactivity levels were similar for all groups (Table 1). In terms of habituation rates, however, all groups were similar except for vehicle-treated sham animals, which showed significantly increased habituation rates [$\chi^2(1) = 6.2$, $P < .05$] in the high relative to the lower luminance open field.

3.2. Experiment 2: effects of acute lorazepam treatment in the high luminance open field and the elevated plus maze

3.2.1. High luminance open field

Fig. 2A shows the total locomotor activity scores of sham and OBX animals treated with vehicle, acutely with 0.1 or 0.5 mg/kg lorazepam or subchronically with 0.1 mg/kg

Table 1
Reactivity ± asymptotic S.E. and habituation ± asymptotic S.E. parameter estimates in both high and lower luminance open-field conditions derived from exponential random effects model fit Behavior = $A \times \exp^{(B \times \text{time})}$, with parameters A and B estimated as random effects

	Reactivity	S.E.	Habituation	S.E.
High luminance open field				
Sham-Veh	8.95	1.410	− 0.0063	0.0017
OBX-Veh	16.18**	1.210	− 0.0066	0.0012
Sham-Flu	10.57	1.460	− 0.0055	0.0017
OBX-Flu	15.35**	1.510	− 0.0112**,††	0.0017
Lower luminance open field				
Sham-Veh	11.16	1.17	− 0.0015	0.0013
OBX-Veh	12.48	1.30	− 0.0059*	0.0014
Sham-Flu	11.66	1.28	− 0.0025	0.0014
OBX-Flu	14.57	1.40	− 0.0078**	0.0015

Likelihood ratio tests were performed to compare parameters between the groups within each open-field condition.
* Significant effect relative to vehicle-treated sham controls at $P < .05$.
** Significant effect relative to vehicle-treated sham controls at $P < .01$.
†† Significant effect relative to vehicle-treated OBX rats at $P < .01$.

lorazepam upon 5 min exposure to the high luminance open field. A two-factor (surgery and drug) independent-groups ANOVA revealed both significant main effect of surgery [$F(1,60) = 22.08$, $P < .01$], demonstrating that OBX animals were hyperactive relative to sham controls. The drug main effect was also significant [$F(3,60) = 3.96$, $P < .05$], but no Surgery × Drug interaction was found. Dunnett's tests revealed that the drug main effect was mainly attributable to the finding that acute administration of 0.1 and 0.5 mg/kg lorazepam significantly decreased locomotor activity ($P < .01$) relative to vehicle treatment. The more modest reduction in activity observed in rats administered subchronic lorazepam (0.1 mg/kg) was not significant.

Fig. 2B shows the time course of the mean locomotor activity for vehicle-treated and lorazepam (0.5 mg/kg)-treated sham and OBX animals, with activity scores totaled at 20 s intervals. Note that in this experiment there were more pronounced differences in the residual rate of responding in the later minutes of the test. Accordingly, we found that a three-parameter model, obtained by including the C-value to account for residual responding, provided significantly better fits than did the two-parameter model. The estimated parameters are presented in Table 2. Vehicle-treated OBX animals were initially more reactive than sham controls [$\chi^2(1) = 6.4$, $P < .05$] but habituated at a comparable rate. Moreover, no significant differences between lorazepam (acute or subchronic)-treated and vehicle-treated sham or lorazepam-treated and vehicle-treated OBX animals were observed for initial reactivity or habituation [χ^2's$(1) < 1.90$, P's$>.05$]. However, as compared to vehicle-treated OBX rats, acute administration of both 0.1 and 0.5 mg/kg lorazepam significantly decreased the residual response rate estimate [$\chi^2(1) = 4.5$ and 8.2, $P < .05$ and .01, respectively]. Subchronic lorazepam administration to OBX animals did not influence this parameter relative to vehicle-treated OBX animals [$\chi^2(1) = 0.6$, $P > .05$]. In sham animals, only the acute 0.5 mg/kg dose of lorazepam significantly reduced the residual response parameter [$\chi^2(1) = 5.1$, $P < .05$].

3.2.2. Elevated plus maze

One sham and two OBX animals treated with acute 0.5 mg/kg lorazepam fell off the maze before the end of the testing session. The data from these animals, therefore, were excluded from all analyses assessing performance in the plus maze.

Fig. 3A shows the total locomotor activity of vehicle-treated and lorazepam-treated sham and OBX animals exposed to a 5 min session of the elevated plus maze. A two-factor ANOVA revealed a significant Surgery × Drug

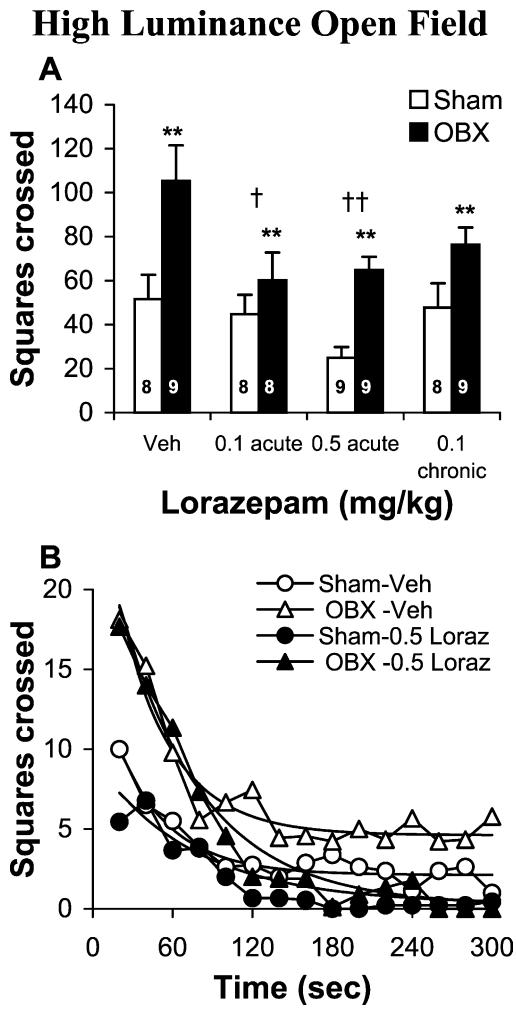

Fig 2. (A) Mean ± S.E.M. number of squares crossed over 5 min in a novel, high luminance open field for sham and OBX rats treated 30 min prior with either vehicle, acute (0.1 or 0.5 mg/kg) lorazepam or subchronic lorazepam (0.1 mg/kg). The number of animals per group is shown at the base of each column. (B) Time course of mean squares crossed in a high luminance open field for vehicle-treated and lorazepam-treated sham and OBX animals, with crossings summed over consecutive 20-s periods. Smooth lines represent fitted exponential models Behavior = $A \times \exp^{(B \times \text{time})} + C$ with parameters A, B and C estimated as random effects. ** Significant main effect at $P < .01$ relative to sham controls. †,††Significant main effect relative to vehicle controls at $P < .05$ and $< .01$, respectively.

Table 2
Reactivity ± asymptotic S.E., habituation ± asymptotic S.E. and residual responding ± asymptotic S.E. parameter estimates in the high luminance open field derived from the exponential random effects model fit Behavior = $A \times \exp^{(B \times \text{time})} + C$

	Reactivity	S.E.	Habituation	S.E.	Residual	S.E.
Sham-Veh	14.97	3.22	−0.0250	0.0067	2.12	0.80
Sham-Lor (0.1) acute	11.84	2.73	−0.0160	0.0053	1.09	0.86
Sham-Lor (0.5) acute	8.74	2.48	−0.0132	0.0050	0.28	0.87
Sham-Lor (0.1) chronic	9.55	2.50	−0.0086	0.0047	0.05	1.30
OBX-Veh	28.16**	3.04	−0.0251	0.0045	4.61*	0.75
OBX-Lor (0.1) acute	25.51**	3.05	−0.0224	0.0044	1.14†	0.81
OBX-Lor (0.5) acute	25.67**	2.58	−0.0161	0.0037	0.15††	0.81
OBX-Lor (0.1) chronic	24.82**	2.73	−0.0196	0.0040	1.91	0.77

All parameters were estimated as random effects. Likelihood ratio tests were performed to compare parameters between the groups.
* Significant effect relative to vehicle-treated sham controls at $P < .05$.
** Significant effect relative to vehicle-treated sham controls at $P < .01$.
† Significant effect relative to vehicle-treated OBX rats at $P < .05$.
†† Significant effect relative to vehicle-treated OBX rats at $P < .01$.

interaction [$F(3,57) = 4.08$, $P < .05$]. Simple effects tests indicated that vehicle-treated OBX animals showed significantly higher total locomotor activity as compared to sham controls ($P < .01$) and that these levels of activity were not affected by subchronic administration of 0.1 mg/kg lorazepam ($P > .05$). Acute lorazepam significantly reduced overall activity at the 0.5 mg/kg dose in shams ($P < .01$) and at both the 0.1 and 0.5 mg/kg doses in OBX animals (P's $< .01$).

Fig. 3B and C show the time spent in the open arms and the percent open-to-total squares crossed in the elevated plus maze, respectively. For open arm time, there was a significant main effect of surgery [$F(1,57) = 5.18$, $P < .05$], as OBX rats tended to spend more time in the open arms relative to shams. Although lorazepam-treated rats tended to spend more time on the open arms, this trend was not reliable, as the main effect of drug and the Drug × Surgery interaction were not significant (F's < 1.0). For percent open-to-total squares crossed, there was both a significant main effect of surgery [$F(1,57) = 10.90$, $P < .01$] and a significant main effect of drug [$F(3,57) = 3.04$, $P < .05$] but no interaction [$F(3,57) = 1.03$, $P > .05$]. The surgery main effect indicated that OBX animals had a higher open-to-total square entry ratio than sham animals. Dunnett's tests revealed that the dose main effect occurred because acute (both 0.1 and 0.5 mg/kg) and subchronic (0.1 mg/kg) lorazepam admin-

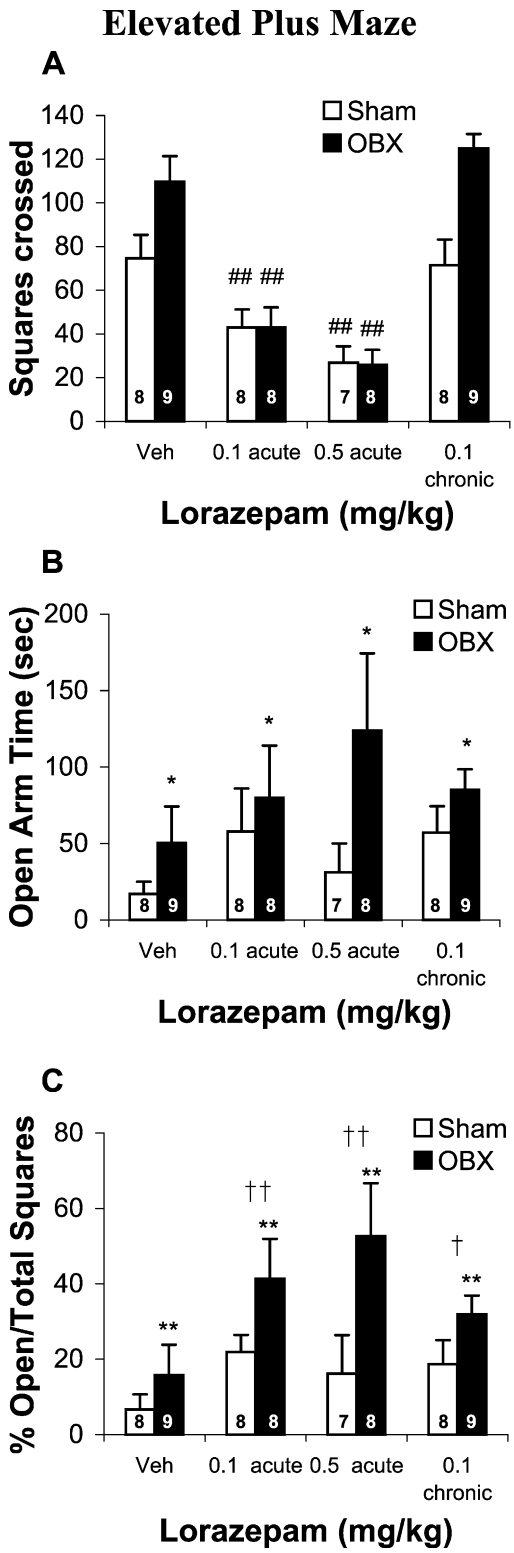

Fig. 3. (A) Mean ± S.E.M. number of squares crossed over 5 min in the elevated plus maze for sham and OBX rats treated 30 min prior with either vehicle, acute (0.1 or 0.5 mg/kg) lorazepam or subchronic lorazepam (0.1 mg/kg). The animals were the same as those used in Fig. 2 (see Materials and methods). (B) Mean ± S.E.M. time spent in the open arms over 5 min for sham and OBX rats treated with either vehicle or lorazepam. (C) Mean ± S.E.M. percent open-to-total squares crossed for sham and OBX rats treated with either vehicle or lorazepam. ##Significant interaction and simple effect at $P < .01$ relative to vehicle-treated rats. *, ** Significant main effect relative to sham controls at $P < .05$ and $< .01$, respectively. †,††Significant Dunnett's test effect relative to vehicle-treated rats at $P < .05$ and $< .01$.

istration significantly increased the ratio of open-to-total arm activity relative to vehicle administration (P's < .05).

4. Discussion

The observation that OBX animals are hyperactive relative to sham-operated animals in a high luminance open field is an old and well-established finding (Kelly et al., 1997; Leonard and Tuite, 1981). The present results, however, suggest that the OBX-induced hyperactivity may be a misnomer, in large part dependent on how the behavior of sham controls varies across environmental conditions. While we found vehicle-treated OBX animals showed increased activity in the high luminance open field, they had reduced activity scores in the lower luminance open field relative to sham-operated controls. Interestingly, the differences between OBX and sham-operated animals across luminance conditions appeared to emerge because sham-operated animals were considerably less active in the high relative to the lower luminance open field. The activity counts were roughly equivalent in vehicle-treated OBX rats across the luminance conditions. The lower activity observed in sham-operated animals in the high luminance (and presumably more aversive) open field might be viewed as an adaptive response. Unlike sham-operated animals, however, vehicle-treated OBX rats appear unable to modify their behavior according to changing environmental circumstances. In the context of the standard high luminance open field, OBX animals seem incapable of inhibiting their behavior to the same extent as shams under aversive conditions.

Indeed, the "hyperactivity" observed in OBX animals has been proposed to arise from increased sensitivity (Jesberger and Richardson, 1986; Van Riezen and Leonard, 1990) or impaired habituation to novelty or stress (Leonard and Tuite, 1981). Using regression modeling, the present results directly examined these proposals and replicated our previous report (Mar et al., 2000) that in the high luminance open field, vehicle-treated OBX rats display a higher level of initial reactivity, but equivalent habituation rates, in comparison to sham-operated animals. However, the reactivity and habituation rate estimates for vehicle-treated OBX rats in the lower luminance condition were comparable to those observed in the high luminance condition. In contrast, reactivity was slightly elevated and habituation rate was greatly reduced in sham-operated animals tested in the lower luminance relative to the high luminance condition. This pattern of results suggests that OBX rats suffer from neither an increased sensitivity nor an impaired rate of habituation to stressful environments. Rather, as suggested above, the behavioral impairment consequent to OBX appears to reflect a failure to adapt appropriately to changing environmental conditions.

As has been reported frequently with a variety of antidepressants (Kelly et al., 1997; Van Riezen and Leonard, 1990), we found that the hyperactivity observed in OBX animals in the high luminance open field can be attenuated by chronic administration of 10 mg/kg fluoxetine (see also Mar et al., 2000). Of interest, these effects were not mediated through a reduction in the initial reactivity estimate but rather through an increase in the rate of habituation. Additionally, fluoxetine administration did not alter overall activity or the reactivity and habituation estimates of OBX rats in the lower luminance condition. These findings suggest that the level of aversiveness or stress of the open field may play an important role both in uncovering a "deficit" in OBX animals and in determining the impact of chronic antidepressant treatment. Antidepressant treatment provided selective beneficial effects by allowing more rapid habituation to a stressful situation. It did not inhibit habituation in the less stressful condition. Accordingly, these results suggest that chronic fluoxetine administration promotes the function of response inhibition mechanisms normally activated by aversive conditions.

Given the close conceptual associations between stress, anxiety and depression intrinsic to most animal models and testing paradigms and the fact that many antidepressants also serve as effective anxiolytics, a reasonable hypothesis emerges whereby antidepressants may be exerting their rectifying effects in OBX animals through anxiolytic mechanisms. We tested this hypothesis using both acute and subchronic administration of the benzodiazepine lorazepam and comparing its effects to that of fluoxetine. We found that although both acute and subchronic lorazepam effectively reduced OBX hyperactivity in the high luminance open field, they did so not by increasing the rate of habituation but by decreasing the residual activity that occurs towards the end of the testing session. In short, although both classes of drugs appeared to promote the adaptive effect of inhibiting the activity of OBX animals in the high luminance open field, the distinct times at which they exerted their effects confirm the operation of different mechanisms of action.

Acute lorazepam administration dose-dependently increased the open-to-total arm ratio in the elevated plus maze. However, whether the increase in open-to-total square ratios can be attributed to an anxiolytic action is unclear. It is well known that lorazepam has sedative properties when administered acutely (File, 1981, 1984), and in the present experiments, this compound dose-dependently reduced the total number of square crossings in the open field and the elevated plus maze in both OBX and sham-operated animals. Accordingly, it is difficult to say whether the reduction in residual responding induced by acute lorazepam is attributable to its anxiolytic or sedative effect. It has been reported that subchronic administration of the benzodiazepine diazepam, which promotes the development of tolerance to the sedative, but not the anxiolytic, effect of benzodiazepines (File, 1981), does not influence open-field activity of OBX rats (O'Connor and Leonard, 1984). We tested this possibility using sham and OBX rats subchronically administered (6–7 days) 0.1 mg/kg lorazepam. We

found that this treatment also increased the ratio of open-to-total square entries (albeit to a lower extent than that observed following acute administration) and yet did not significant reduce overall activity (i.e., total square entries) in the plus maze or the open field. Thus, subchronic lorazepam administration permitted at least a partial dissociation between sedative and anxiolytic effects. Of interest, although subchronic lorazepam administration also tended to reduce the residual response estimate (*C*-parameter), the magnitude of this reduction was smaller than that observed following acute administration (and not statistically significant). Thus, it may be that the reduction in the residual response rate observed following acute lorazepam may be the result of a sedative effect or a combined sedative/anxiolytic effect. It is worth noting that, independent of whether the effects of lorazepam are the result of an anxiolytic or sedative action, the mechanism mediating the activity-reducing effect of fluoxetine in the high illumination open field was temporally distinct from the mechanism underlying that provoked by lorazepam (i.e., both acute and subchronic lorazepam did not affect habituation rate).

In conclusion, chronic administration of fluoxetine (10 mg/kg) promoted a more rapid habituation to a novel open field in OBX animals that was dependent upon the degree of aversiveness of the open field. This effect was likely not the result of an anxiolytic action, as the effects evoked by lorazepam were temporally distinct from those induced by fluoxetine. Exposure to stress has been associated with the onset, severity and susceptibility to relapse of a depressive episode (Anisman and Zacharko, 1990; Post, 1992). Accordingly, the present results suggest that antidepressants may exert their therapeutic effects and/or protect against relapse by promoting more adaptive coping responses to stress.

References

Ader R. Adrenocortical function and the measurement of emotionality. Ann NY Acad Sci 1969;159:791–805.

Alberts JR, Friedman MI. Olfactory bulb removal but not anosmia increases emotionality and mouse killing. Nature 1972;238:454–5.

Anisman H, Zacharko RM. Multiple neurochemical and behavioral consequences of stressors: implications for depression. Pharmacol Ther 1990; 46:119–36.

Araki H, Yamamoto T, Watanabe S, Ueki S. Changes in sleep–wakefulness pattern following bilateral olfactory bulbectomy in rats. Physiol Behav 1980;24:73–8.

Caccia S, Cappi M, Fracasso C, Garattini S. Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. Psychopharmacology 1990;100:509–14.

Cain DP, Paxinos G. Olfactory bulbectomy and mucosal damage: effects on copulation, irritability, and interspecific aggression in male rats. J Comp Physiol Psychol 1974;86:202–12.

Cairncross KD, Cox B, Forster C, Wren A. A new model for the detection of antidepressant drugs: olfactory bulbectomy in the rat compared with existing models. J Pharmacol Methods 1978;1:131–43.

Cairncross KD, Cox B, Forster C, Wren AF. Olfactory projection systems, drugs and behavior: a review. Psychoneuroendocrinology 1979a;4: 253–72.

Cairncross KD, Wren A, Forster C, Cox B, Schnieden H. The effect of psychoactive drugs on plasma corticosterone levels and behaviour in the bulbectomized rat. Pharmacol, Biochem Behav 1979b;10:355–9.

Dalley JW, Mason K, Stanford SC. Increased levels of extracellular noradrenaline in the frontal cortex of rats exposed to naturalistic environmental stimuli: modulation by acute systemic administration of diazepam or buspirone. Psychopharmacology 1996;127:47–54.

Edwards DA. Non-sensory involvement of the olfactory bulbs in the mediation of social behaviour. Behav Biol 1974;11:287–302.

File SE. Rapid development of tolerance to the sedative effects of lorazepam and triazolam in rats. Psychopharmacology (Berlin) 1981;73: 240–5.

File SE. Behavioural pharmacology of benzodiazepines. Prog Neuro-Psychopharmacol Biol Psychiatry 1984;8:19–31.

Gardier AM, Lepoul E, Trouvin JH, Chanut E, Dessalles MC, Jacquot C. Changes in dopamine metabolism in rat forebrain regions after cessation of long-term fluoxetine treatment: relationship with brain concentrations of fluoxetine and norfluoxetine. Life Sci 1994;54:51–6.

Giardina WJ, Radek RJ. Effects of imipramine on the nocturnal behavior of bilateral olfactory bulbectomized rats. Biol Psychiatry 1991;29: 1200–8.

Hirsch JD. The neurochemical sequelae of olfactory bulbectomy. Life Sci 1980;26:1551–9.

Igarashi E, Takeshita S. Effects of illumination and handling upon rat open field activity. Physiol Behav 1995;57:699–703.

Jesberger JA, Richardson JS. Effects of antidepressant drugs on the behavior of olfactory bulbectomized and sham-operated rats. Behav Neurosci 1986;100:256–74.

Kelly JP, Wrynn AS, Leonard BE. The olfactory bulbectomized rat as a model of depression: an update. Pharmacol Ther 1997;74:299–316.

La Rue CG, Le Magneu J. The olfactory control of meal pattern in rats. Physiol Behav 1972;9:817–21.

Leonard BE, Tuite M. Anatomical, physiological and behavioral aspects of olfactory bulbectomy in the rat. Int Rev Neurobiol 1981;22:251–86.

Mar A, Spreekmeester E, Rochford J. Antidepressants preferentially enhance habituation to novelty in the olfactory bulbectomized rat. Psychopharmacology 2000;150:52–60.

Marks HE, Remley NR, Seago JD, Hastings DW. Effects of bilateral lesions of the olfactory bulbs of rats on measures of learning and motivation. Physiol Behav 1971;7:1–6.

McNish KA, Davis M. Olfactory bulbectomy enhances sensitization of the acoustic startle reflex produced by acute or repeated stress. Behav Neurosci 1997;111:80–91.

Meguid MM, Gleason JR, Yang Z-J. Olfactory bulbectomy in rats modulates feeding pattern but not total food intake. Physiol Behav 1993;54: 471–5.

O'Connor WT, Leonard BE. Effect of stress on the secretion of corticosterone in the bulbectomized rat model of depression. Irish J Med Sci. 1984;226.

Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.

Post RM. Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. Am J Psychiatry 1992;149:999–1010.

Primeaux SD, Holmes PV. Role of aversively motivated behavior in the olfactory bulbectomy syndrome. Physiol Behav 1999;67:41–7.

Sakurada T, Shima K, Tadano P, Sakurada S, Kisara K. Sleep–wakefulness rhythms in the olfactory bulb lesioned rats. Jpn J Pharmacol 1976; 26:97P.

Seliger DL. Effects of age, sex, and brightness of field on open-field behaviors of rats. Percept Mot Skills 1977;45:1059–67.

Sieck MH. The role of the olfactory system in avoidance learning and activity. Physiol Behav 1972;8:705–10.

Sieck MH, Baumbach HD. Differential effects of peripheral and central anosmia producing techniques on spontaneous behavior patterns. Physiol Behav 1974;13:407–25.

Stockert M, Serra J, de Robertis E. Effect of olfactory bulbectomy and

chronic amitryptiline treatment in rats. ^{3}H-imipramine binding and behavioral analysis by swimming and open field tests. Pharmacol, Biochem Behav 1988;29:681–6.

Van Riezen H, Leonard BE. Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. Pharmacol Ther 1990;47:21–34.

Van Riezen H, Schnieden H, Wren A. Behavioral changes following olfactory bulbectomy in rats: a possible model for the detection of antidepressant drugs. Br J Pharmacol 1976;57:426P–7P.

Williams DI. Maze exploration in the rat under different levels of illumination. Anim Behav 1971;19:365–7.