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The mouse light/dark box test

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Abstract

The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light. The test apparatus consists of a small dark safe compartment (one third) and a large illuminated aversive compartment (two thirds). The test was developed with male mice. The strain, weight and age may be crucial factors. The extent to which an anxiolytic compound can facilitate exploratory activity depends on the baseline level in the control group. Differences between the type and severity of external stressors might account for the variable results reported by different laboratories. The light/dark test may be useful to predict anxiolytic-like or anxiogenic-like activity in mice. Transitions have been reported to be an index of activity-exploration because of habituation over time, and the time spent in each compartment to be a reflection of aversion. Classic anxiolytics (benzodiazepines) as well as the newer anxiolytic-like compounds (e.g. serotonergic drugs or drugs acting on neuropeptide receptors) can be detected using this paradigm. It has the advantages of being quick and easy to use, without requiring the prior training of animals.

Keywords: Antidepressant; Antipsychotic; Anxiolytic; Light/dark test, mouse; Neuropeptide receptor ligand

1. Introduction

The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light (Crawley and Goodwin, 1980). A natural conflict situation occurs when an animal is exposed to an unfamiliar environment or novel objects. The conflict is between the tendency to explore and the initial tendency to avoid the unfamiliar (neophobia). The exploratory activity reflects the combined result of these tendencies in novel situations. Thus, in the light/dark test, drug-induced increase in behaviours in the white part of a two-compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity. An increase in transitions without an increase in spontaneous locomotion is considered to reflect anxiolytic activity. It is interesting to note that this effect is only observed in certain strains of mice or with certain drugs. This model differs from other purported models of anxiety which are not equivalent in terms of elicited/induced emotional state (File, 1992; Njung'e and Handley, 1991; Treit, 1990; De Vry et al., 1993). The goal of this paper is to review the main data obtained with the light/dark test and point out the inconsistent findings linked with the various modifications of the apparatus as well as the methodology.

2. Test apparatus

Although the light/dark test was based on the initial model described by Crawley and Goodwin (1980), many authors have used it with several structural modifications (Table 1).

Typical dimensions of the compartment are generally one third for the dark compartment and two thirds for the light compartment with an exterior size of $46 \times 27 \times 30$ cm ($l \times b \times h$). Nevertheless, Costall et al. (1989) have differently distributed the compartments with two thirds for the dark compartment. The model is based on the observation that although nocturnal rodents such as mice will naturally tend to explore a novel environment, open fields appear to have aversive properties which inhibit exploratory behaviour. Here, the safe area is the small dark compartment (one third) and the aversive area is the large illuminated compartment

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Table 1
The main different light/dark procedures used

References	Crawley and Goodwin, 1980; Crawley and Davis, 1982; Crawley et al., 1983; Crawley et al., 1984, 1997	Costall et al., 1988a,b, 1989, 1990, 1991, 1993	Gao and Cutler, 1992	Belzung et al., 1987, 1994	Hascoët and Bourin et al., 1996; Hascoët and Bourin, 1998; Hascoët et al., 1999, 2000a,b	Shimada et al., 1995	Young and Johnson, 1988, 1991a,b
Size of boxes (cm)	44 × 21 × 21 1/3 dark 2/3 light	45 × 27 × 27 2/5 dark 3/5 light	31 × 46 (dark) 60 × 46 (light)	20 × 20 × 14 1/2 dark 1/2 light tunnel between the two compartments	46 × 27 × 30 1/3 dark 2/3 light	corridor-type runway two compartments diagonally placed on corner	40 × 33 × 20 (dark) 42 × 42 × 20 (light)
Light/dark cycle	normal	inversed	inversed	inversed	normal	normal	4 h of dark before testing
Compartment where mice are placed	in the light	in the light	in the light	in the light	in the light	in the corner	in the light
Duration of the test	10 min	5 min	5 min	5 min	5 min	3 min	10 min
Parameters used							
Latency time		+			+		+
Transitions	+	+	+	+	+		+
Movements in the light	+	+	+		+	+	+
Movements in the dark	+	+	+		+	+	+
Time in the light	+	+	+	+	+	+	+
Time in the dark	+	+	+	+	+	+	+
Rears		+		+		+	+

"+" parameters used by authors considered.

(two thirds). The opening between the two compartments is not more than 7 cm. One variation in the model was the size of the compartment (Young and Johnson, 1991a,b) with an increase to 42 × 42 × 30 cm for the light compartment and 40 × 23 × 20 cm for the dark one. Gao and Cutler (1992) used an apparatus with other dimensions: 31 × 46 cm for the dark area and 60 × 46 for the light one. Imaizumi and Onodera (1993) have used a modified model based on the automated one of Young and Johnson (1991a,b) with slight modifications. The test chamber consisted of two compartments of equal size (15 × 15 × 15 cm) with two partitions between the compartments. Another modified model is the one used by Belzung et al. (1987). The apparatus consisted of two polyvinylchloride boxes of the same size (20 × 20 × 14 cm). One was darkened with cardboard and the other was brightly illuminated; an opaque plastic tunnel (5 × 7 × 10 cm) separated the two compartments. The more modified light/dark transition test is the one of Shimada et al. (1995) as the apparatus was a corridor-type runway, with outer (40 × 12 cm) and inner (20 × 12 cm) walls. Two rectangular chambers made of black Plexiglas were diagonally placed at the corner of the corridor. The centre of the apparatus was illuminated by a fluorescent lamp.

In the first descriptions of the light/dark test in which anxiolytics were used in mice, photocells across the partition were used to detect transitions between the two areas (Crawley, 1981, 1985; Crawley and Goodwin, 1980). Later studies used human observers to record transitions of the mice and the amount of time mice spent in each area (Belzung et al., 1987). Video recordings of mice behaviour in the chamber have also been used to detect an increase in locomotor and rearing activities in the illuminated area accompanied by a decrease in these behaviours in the dark area (Crawley, 1981; Blumstein and Crawley, 1983). An apparatus was developed that allows automatic measurement of locomotion, rearing and time spent in light and dark zones and shuttle crossings between zones, using computer-controlled detection equipped with infrared beam sensors by which interruptions of the infrared beams in the chamber are automatically recorded by an analyser and then transmitted to a computer (Young and Johnson, 1991a,b). The computerised apparatus is highly appropriate because the animals are subjected to minimal external perturbations.

The apparatus used by Hascoët and Bourin (1998) consisted of a fully automated box monitored by computer. An open-topped rectangular box (46 × 27 × 30 cm high) was divided into a small (18 × 27 cm) area and a large (27 × 27 cm) area with an opening door (7.5 × 7.5 cm) located in the centre of the partition at floor level. The small compartment was painted black and illuminated by a dim red light (60 W; 4 lx), whereas the large compartment was painted white and brightly illuminated with a 60-W (400 lx)

light source. The compartments were equipped with infrared beam sensors (four in the white area, three in the black one), thereby enabling the detection of locomotion in each zone, time spent in each zone, latency of the first crossing from one compartment to the other, and shuttle crossings between both compartments. The data for these four parameters were directly collected by computer.

3. Animals

This test was developed with male mice. As inbred mice displayed substantial variability in spontaneous behaviour, the choice of strain may be a crucial parameter. Early studies with this paradigm concluded that highly active strains of mice show consistently larger percentage increases in exploratory behaviour after diazepam treatment. The C57Bl/6J and the SW-NIH strains seem to be the strains of choice for anti-anxiety testing in the light/dark test (Crawley and Davis, 1982).

The C57Bl/6J strain has been reported to show a robust effect, as demonstrated by a maximum diazepam response of 129% in mean exploratory behaviour (Crawley and Davis, 1982). Strains with a low number of baseline transitions generally show weak responses to anxiolytics (Crawley et al., 1997). However, the results of a recent study call this supposition into question (Hascoët and Bourin, 1998). C57Bl/6J mice were obtained from two different sources and tested in comparison with the Swiss mouse strain. All strains showed the same baseline transition activity. Swiss mice showed a decrease in the time spent in the dark area after 1 mg/kg of diazepam (46%), as did the C57Bl/6J mice from IFFA CREDO; however, this effect was less significant. On the other hand, C57Bl/6J mice from Janvier breeding farm (Le Genest, France) did not show any significant anxiolytic behaviour at any dose. All strains showed a similar baseline activity in movements in each compartment. These results demonstrate that the Swiss strain does display anxiolytic-like behaviour, and that the activity is stronger than that observed with the C57Bl/6J strain, but probably we need to be careful with the origin of the strain. The light/dark exploration test has not been validated for female mice and the influence of the oestrous cycle has not been investigated.

Another parameter that must be discussed in interpreting the results is the weight, or more precisely, the age and the neuronal maturation of the animals. Some authors (Pletnikov et al., 1996), using the test of habituation of the acoustic startle response and freezing responses in rats of different ages, found that rats were not able to express long-term habituation of the acoustic startle response before 30 days of age. The question of age seems therefore to be of particular importance for experimental conditions. Male Swiss mice of different ages (from 3.5 to 8 weeks; 14–32 g) were used in the light/dark test, receiving saline only and their performance was compared. The age at which control values were optimum was 4 weeks (Hascoët et al., 1999). Mice of this age spent 58% of the total test duration in the dark compartment. The oldest mice (i.e. 8 weeks old) exhibited an increase in total activity, characterised by increase in movements in each compartment, together with an increase in the number of transitions. An age-related effect was found, the best period being that of 4 weeks, suggesting that the results of mice in the light/dark paradigm should be interpreted with caution. It is now well established that the diminished central nervous system function that occurs with aging is accompanied by discrete changes in various neurotransmitter networks and/or receptors. Problems of methodology are also highlighted in that increasing weight and age should be taken into consideration in chronic studies or repeated administration studies where animals are retained for several weeks.

4. Test conditions

The extent to which an anxiolytic compound can facilitate exploratory activity depends on the baseline level in the control group. There are a number of non-genetic non-pharmacological manipulations that modulate the general stress levels of animals, which, when performed before testing, have profound effects on behaviour. Deliberate or accidental manipulation of these influential factors can also dramatically alter the effects of drugs (Hoggs, 1996). Differences between the type and severity of external stressors (housing conditions, handling procedures during test) might account for variable results reported by different laboratories, because stress affects motor activity, and different types of stressors might affect the behavioural response differently (Sanchez, 1996).

Each new drug is first screened for nonspecific increases or decreases in general locomotion in a small bare undifferentiated arena in an activity metre which measures all forms of horizontal and vertical locomotor activity. Any drug that increases both transitions and locomotion is considered a general motor stimulant and is regarded as a putative anxiolytic. Studies suggest that acute stress immediately before testing mice in a black and white two light/dark compartment test box can enhance the anxiolytic-like response and thereby increase the robustness of the test model. A study by Belzung and Le Pape (1994) suggests that the tail suspension test compared with the forced swimming test and the foot shock test is an appropriate stressor, producing a general behavioural suppression in both compartments, with the administration of diazepam producing a selective disinhibition of behaviour in the white compartment. Tail suspension test as an acute stress immediately before the test can significantly increase the sensitivity to anxiolytic-like responses (Belzung and Le Pape, 1994).

In the procedures of Crawley and Goodwin (1980) and Costall et al. (1989), faeces are removed after a trial and urine is wiped up and the box is cleaned with water. To reduce any neophobic response to the test situation in the

Hascoët and Bourin (1998) procedure, the light/dark compartments are first doiled by mice other than those used during the test. Mice are always tested in a soiled apparatus and there is no cleaning between trials. Results have demonstrated that cleaning between trials masks the anxiolytic behaviour observed with 1 mg/kg of diazepam in a soiled apparatus. It is suggested that measures then better reflect the influence of dark or bright areas on exploration activity rather than the influence of a clean new area smelling of detergent. A soiled apparatus removed, or at least reduced, the neophobia factor; the aversive stimulus is the novel environment.

In the majority of experiments, mice are taken from a dark holding room in a dark container to the dark testing room where, after more than a 1-h period of adaptation to the new environment, they are placed in the test box. In most experiments, mice are placed in the centre of the white, brightly lit area and the operator withdraws from the room. If mice are removed from the holding room during their dark cycle in a dark container to the dark test room and placed in the test box where both sections are illuminated with a red light, they demonstrate random activity in the two compartments. Thus, mice spent approximately 60–70% of their time in the white area and 30% in the black area, which appears to reflect the relative size of the two chambers. This pattern of exploratory behaviour continued if red illumination in the white section was replaced with a relatively low level of white illumination of 10–240 lx. However, 400-lx illumination in the white area was sufficiently aversive to significantly reduce the time spent and the reavings and line crossings in the white section, with corresponding increases in the black. Furthermore, the time taken for the initial movement of mice from the white section to the black was reduced by 50%; conversely, animals placed initially in the black section delayed their movement into the white section (Costall et al., 1989).

It has been documented that many types of behaviour in nocturnal rodents show a circadian variation with a higher activity during the dark period and a lower activity during the light period (Górka and Maj, 1986). The diurnal rhythm of the animals is thus an important factor. Therefore, the effects of cycle reversal and exposure to the apparatus on selected physiological measures have been investigated. Results (Onaivi and Martin, 1989; Crawley, 1985) suggest that reversal of the light/dark cycle is critical for exploratory behaviour in this test because behavioural changes are affected by plasma corticosterone levels, with the greatest sensitivity occurring during the dark cycle. Animals are maintained on a reversed 12:12 light/dark cycle and are adapted to the reversed light/dark cycle for at least 2–3 weeks before the commencement of drug administration and anxiety testing.

In the mouse light and dark habituation test (Costall et al., 1989), with repeated daily testing, mice habituate to the paradigm such that they ‘‘learn’’ to locate the position of the opening which allows movement from the aversive white section to the dimly lit dark section in which they spend more time and exhibit most exploratory behaviour. Consequently, they display a reduced latency to move from the white to the black section of the test box (Barnes et al., 1990). The crucial factor is whether the environment is sufficiently novel to elicit exploration and this may to some extent be strain and apparatus specific. Naive mice were tested in six trials at 2- to 3-day intervals over a 2-week period by Blumstein and Crawley (1983), to determine the effects of multiple use of animals and to ascertain the test–re-test reliability of the protocol and also to determine whether or not learning occurred. They found that group-housed mice could be routinely used repeatedly up to three times and that the interval between treatments and time of day did not appear to be critical when designing the test protocol. Repeated testing of 12 naive mice placed individually in the centre of the white chamber and assessment of exploratory measures of anxiety were undertaken by Onaivi and Martin (1989). They found that daily repeated testing was possible with a maximum of up to four times a week using naive mice. Barry et al. (1987) observed habituation to the test environment and Costall et al. (1989) also found that, on daily testing, naive young adult animals habituated to the test system when compared to naive aged mice.

5. Scoring of behaviour

Crawley and Goodwin (1980) described a model in which benzodiazepines produced a facilitation of exploratory behaviour between an illuminated open field and a dark enclosure. Mice placed in the white area (which they found aversive) would generally move around the periphery until they found an opening, at floor level, to enable access to the black compartment, and this usually occurred within 7–12 s. The essential feature was the measurement of increased transitions between the light and dark chambers, the time spent in each compartment remaining the same. Costall et al. (1989) found that increased exploratory behaviour was associated with an increased time spent in the illuminated area, transitions between the two compartments remaining unchanged. Exploratory activity depends on the baseline level in the control group and behavioural data suggest that naive mice prefer the dark chamber, where they spend approximately 60% of their time. Entry into a chamber is defined as the placement of all four paws in the chamber. A correlation test performed between light and dark transitions and exploratory behaviours of mice in the illuminated side of the two chamber apparatus showed a significant correlation between the number of exploratory rearings, defined as directed sniffing with the forepaws extended vertically upon the sides of the chamber, and the number of light/dark transitions (Crawley et al., 1984).

Anxiolytics have been found to increase locomotion and time spent in the light zone, whereas anxiogenics decrease them (Imaizumi et al., 1994a,b), and mice placed in the

brightly lit white area show a reduced latency in moving into the black section, an increase in time spent in the black section, with markedly increased rearings and line crossings in this area, whereas all such measures are markedly decreased in the white section (Shimada et al., 1995). A parameter suggested by Lapin (1999) as an index of the effect of anxiogenics is the leaning out (or peeking-out) of the dark chamber by the mice, where a decrease in the rate of leaning out appeared to be a constant effect of standard anxiety-inducing drugs.

A disinhibition of the suppression of behaviour is shown by the time taken for mice to move from or to the white section. Thus, control mice placed in the brightly lit white section would move rapidly into the black area: mice placed in the black area would show a delay in moving into the white. After drug treatment, the apparent apprehension of remaining in or moving to the white area was abolished, and the delay for the animals to move from the white section, or the speed of moving from the black section, showed that aversion was reduced below that of normal mice. It has been suggested that putative "exploratory" activity measured in this paradigm is not simply a generalised motor effect, but rather is a function of the novelty of converting environments with different characteristics. A study by Griebel et al. (1997a) included the parameters time spent in the lit box, attempt at entry into the lit box followed by avoidance responses, which includes stretched attend postures (the mouse stretches forward and retreats to original position) and total number of tunnel crossings. Diazepam increased the time spent and decreased the number of attempts. A diminished number of aborted attempts to enter the aversive area is a profile which is consistent with an anxiolytic-like action (Griebel et al., 1996b).

Increases in behavioural activity in the lit area are not due to a generalised increase in motor behaviour because total activity remains unchanged. Interesting results were obtained with the pure psychostimulant adrafinil (Imaizumi et al., 1994a,b; Costall et al., 1993) and the stimulant antidepressant amineptine (Costall et al., 1989). No change in latency time was noticed. Enhanced transitions and movements in both compartments were noted. The psychostimulant effect did not induce any increase in the time spent in the dark compartments showing that this parameter is specific for anxiolytic activity. The stimulant profile of action of adrafinil and amineptine in the black and white test is different from that of amphetamine, which is an anxiogenic psychostimulant.

From data collected in the literature, it is difficult to compare the effects of movements in each compartment. Indeed, movements were expressed as the time spent in the compartment under consideration. However, it seems obvious that mice that spend less time in one compartment demonstrate few movements and vice versa. It was therefore surprising to see false sedative effects or false psychostimulant effects. To avoid this problem, in a recent study (Hascoët and Bourin, 1998), results of movements/exploratory behaviour in each area were expressed as a function of the time spent in the compartment under consideration. This approach resulted in a more reliable idea of the indices of exploration and made the comparison between treatments easier.

Another parameter, the latency time for the first passage from the light compartment to the dark one, has been used by some other authors (Costall et al., 1989, 1990, 1993). The real sense of this parameter is difficult to appreciate and is rarely discussed in the literature. Two hypotheses could be advanced. An increase in latency time could be the result of disinhibitory behaviour and decreased anxiolysis, by which animals spend more time exploring the white area. The other explanation is the influence of sedation, where animals are unable to move quickly to the dark compartment (Rodgers and Sheperd, 1993). Data suggest that the time mice spend in the lit area and behavioural activities such as locomotor and rearing behaviours may be more useful measures of the anxiolytic potential of a compound than transitions between the two compartments. In fact, the measurement found to be most consistent and useful for assessing anxiolytic-like action is the time mice spend in the lit area, because this parameter provides the most consistent dose–effect results with drugs (Hascoët and Bourin, 1998).

In the light/dark test, a range of active or passive behaviours, such as freezing, thigmotaxis and risk assessment, are almost invariably ignored in favour of a simple spatiotemporal index (Rodgers, 1997).

6. Drugs

In the present section, the results were obtained after systemic administration of the drugs by the intraperitoneal or subcutaneous route (Tables 2–7).

6.1. Effects of ligands acting on benzodiazepine sites

Many animal models of anxiety have been developed on the basis of their sensitivity to benzodiazepines. Benzodiazepines are reliably detected in this paradigm (Table 2), as in many other animal models of anxiety. However, high doses can induce sedation detectable by a significant decrease in transitions (Crawley, 1981).

Some partial agonists are also active in the test but not the antagonist flumazenil. The inverse agonists are mainly anxiogenic.

6.2. Effects of drugs acting on 5-HT$_1$ receptors

The recent introduction to clinical practice of the non-benzodiazepine anxiolytic buspirone and the discovery of new selective compounds have resulted in a resurgence of the study of the involvement of serotonin in anxiety (Table 3). The classic 5-HT hypothesis of anxiety suggests that decreased anxiety is related to decreased activity in control 5-HT neurons and vice versa; however, paradoxical drug

Table 2
Effects of ligands acting on the BZD sites in the light/dark test in mice acute administration

Drugs	Effects	Authors
Benzodiazepines (BZD)		
Diazepam	++	Crawley, 1981; Hascoët and Bourin, 1998; Onaivi and Martin, 1989; Young and Johnson, 1991b; Costall et al., 1990; Imaizumi et al., 1994a,b
Flurazepam	++	Crawley, 1981
Lorazepam	++	De Angelis, 1992
Alprazolam	++	Shimada et al., 1995; Hascoët and Bourin, 1998
Chlordiazepoxide	++	Crawley, 1981; Kilfoil et al., 1989
RO-4864 (peripheral)	0	Crawley, 1981
BZD partial agonists		
Y 23684	++	Yasumatsu et al., 1994
RO 16-6028	++	Belzung et al., 1989
CGS 9896	++	Smith and Crawley, 1986
BZD antagonists		
Flumazenil	0	Crawley, 1985; Hascoët and Bourin, unpublished; Shimada et al., 1995; Belzung et al., 1987
CGS-8216	0	Crawley, 1985; Wieland et al., 1991
BZD inverse agonists		
FG 7142	0	Crawley et al., 1984; Kilfoil et al., 1989; Hascoët and Bourin, 1998
B CCM	– –	Crawley et al., 1984; Imaizumi et al., 1994a,b
B CCE	– –	Shimada et al., 1995; Imaizumi et al., 1994a,b
B CCP	– –	Onaivi and Martin, 1989

" + " anxiolytic-like effects; " – " anxiogenic-like effects; "0" no effect.

effects have often been found. A great number of studies found no evidence for anxiolytic- or anxiogenic-like effects of drugs that modulate 5-HT neurotransmission (Griebel, 1995). The reasons for this variability in drug effect remain in part unknown, but certainly include factors such as species difference, sex of animals, and environment in which a test is conducted. Variations in effects might also reflect differences in the degree to which the models themselves represent fear or anxiety (Barret and Vanover, 1993; Handley, 1995; Handley and McBlane, 1993). The most convincing explanation of these discrepancies has recently emerged from several papers by Handley (1995), Handley and McBlane (1993) and Handley et al. (1993) who suggested that there is more than one 5-HT mechanism involved in anxiety models. It is obvious that the various models are not equivalent. Thus, models based on spontaneous responses, such as exploration tests like the light/dark test, may reflect a type of anxiety linked with uncontrollable stress ("depressive anxiety") because animals are exposed by force to a novel and/or aversive environment from which they cannot escape, while those based on conditioning (e.g.

Table 3
Effects of ligands acting on the serotonin system in the light/dark test in mice acute administration

Drugs	Effects	Authors
5-HT$_{1A}$ receptor agonists		
Buspirone	++	Crawley, 1981; Hascoët and Bourin, 1998; Onaivi and Martin, 1989; Young and Johnson, 1991b; Costall et al., 1989; Imaizumi et al., 1994a,b
8-OH-DPAT	++, 0	Lopez-Rubalcava et al., 1992; Hascoët and Bourin, unpublished data
Gepirone	0	Bill et al., 1989
Ipsapirone	++	Bill et al., 1989
MDL 73005EF	++	Bill et al., 1989; Misslin et al., 1990
Flesinoxan	++	Schipper et al., 1991
5-HT$_{1A}$ receptor antagonists		
Nan 190	0	Hascoët and Bourin, unpublished data
Pindolol	0	Fernandez-Guasti and Lopez-Rubalcava, 1990; Lopez-Rubalcava et al., 1992
5-HT$_{1B}$ receptor agonists		
Anpirtoline	++	Metzenauer et al., 1992; Hascoët and Bourin, unpublished data
5-HT$_{2}$ receptor agonists		
mCPP	0	Griebel et al., 1991; Nic Dhonnchadha et al., in press
DOI	0, – –	Onaivi and Martin, 1989; Young and Johnson, 1991a,b; Nic Dhonnchadha et al., in press
RO 60-0175	0	Nic Dhonnchadha et al., in press
BW 723C86	0	Nic Dhonnchadha et al., in press
5-HT$_{2A}$ receptor antagonists		
SR 46349B	0	Nic Dhonnchadha et al., in press
5-HT2$_{a/c}$ receptor antagonists		
Ritanserin	+, 0, – –	Costall et al., 1988a; Gao and Cutler, 1993; Barnes et al., 1992a
Ketanserin	–	Nic Dhonnchadha et al., in press
5-HT$_{2c}$ receptor antagonists		
RS 10-2221	0	Nic Dhonnchadha et al., in press
SDZ SER082	0	Nic Dhonnchadha et al., in press
SB 206553	0	Nic Dhonnchadha et al., in press
5-HT$_{3}$ receptor agonists		
2-methyl-5-HT	0	Costall et al., 1988b; Hascoët and Bourin, unpublished data
5-HT$_{3}$ receptor antagonists		
Ondansetron	+, 0	Costall et al., 1988b, 1989; Tyers et al., 1987; Young and Johnson, 1988; Hascoët et al., unpublished data
MDL 72 222	+, 0	Onaivi and Martin, 1989; Bill et al., 1992; Costall et al., 1989
Zacopride	+	Young and Johnson, 1991b; Barnes et al., 1992b
DAU 6215	+	Borsini et al., 1993
Granisetron	+	Costall et al., 1989; Barnes et al., 1992a

" + " anxiolytic-like effects; " – " anxiogenic-like effects; "0" no effect.

Vogel's conflict test) may reflect a type of anxiety associated with controllable aversive events (''anticipatory anxiety'').

6.3. Effect of acute administration of 5-HT_2 receptor agonists

[(±)-2,5-Dimethoxy-4-iodoamphetamine] (DOI) significantly reduced the movements in the dark compartment at doses of 2, 4 and 8 mg/kg after i.p. acute administration, and the latency time for passing from the lit compartment to the dark compartment at doses of 1 and 8 mg/kg (Table 3). BW 723C86 had no effect on mouse behaviour in the light/dark paradigm at the doses tested. RO 60-0175 significantly reduced transitions between compartments and movement in the lit compartment at the dose at 4 mg/kg. [1-(3-Chlorophenyl)piperazine] (mCPP) induced a significant decrease in the movements in the lit compartment at the dose of 0.5 mg/kg.

6.4. Effect of acute administration of 5-HT_2 receptor antagonists

Of the indices of anxiety measured (transitions and percentage time spent in the dark compartment), only ketanserin administration induced a reduction in the number of transitions between compartments along with a significant reduction in the movements in the dark compartment (Table 3). Neither RS 10-2221 SDZ SER082 nor SB 206553 administration had an effect on mouse behaviour at all doses tested in the light/dark paradigm. SR 46349B administration significantly increased movement in the dark compartment. Thus, ketanserin administration induced an anxiogenic-like effect, while the specific 5-HT_{2A} receptor antagonist SR 46349B was without an effect in this paradigm. The effects observed with ketanserin may potentially involve adrenergic or histaminergic receptor activity (Kennett et al., 1994) or the combined antagonism of both 5-HT_{2A} and 5-HT_{2C} receptors. The literature has provided little evidence of anxiolytic-like effects of 5-HT_2 receptor ligands in the mouse light/dark test (Cheng et al., 1994; Sanchez, 1995; Costall and Naylor, 1995, 1997; Griebel et al., 1997b), perhaps indicating that the 5-HT_2 receptor is not implicated in this test and thus that the model is incapable of detecting the potential anxiolytic-like effects of 5-HT_2 receptor antagonists. It has been suggested that the 5-HT_3 receptor rather than the 5-HT_2 receptor may be involved in the fear provoked by the light/dark paradigm, and that the nucleus accumbens (Higgins et al., 1991) or the amygdala (Stefanski et al., 1993) may be involved in mediating the disinhibitory effects of 5-HT_3 receptor antagonists.

6.5. Effects of acute administration on 5-HT3 receptors

Anxiolytic-like effects of 5-HT_3 receptor antagonists have been established in selected test procedures (Kilpatrick et al., 1990) (Table 3). In these, only the light/dark test choice paradigm in mice constantly showed an anxiolytic-like action of these agents (Griebel, 1995).

6.6. Effects of acute administration of antidepressants

A growing interest in the use of antidepressant drugs for the treatment of anxiety disorders has led to study of their potential anxiolytic-like effects in various animal models (Table 4).

Data suggest that moclobemide, a reversible inhibitor of type A monamine-oxidase enzyme, significantly reduces anxiogenic-like behaviour in the light/dark test, whereas selegiline, an irreversible and selective monoamineoxydase-B inhibitor, shows a lack of anxiolytic-like effect (De Angelis and Furlan, 2000). Animal studies and clinical findings suggest that serotonin selective reuptake inhibitors, when given acutely, do not reduce experimental anxiety as the symptoms of general anxiety disorder and panic disorders (Bodnoff et al., 1989; Lightowler et al., 1994; Matto et al., 1995), which are in contrast to results found by Hascoët et al. (2000b) (Table 4). Indeed, studies have frequently reported that acute administration of SSRIs elicits anxiogenic-like responses (File et al., 1985; Bodnoff et al., 1989). Sanchez and Meier (1997), who studied the profile of five SSRIs, found that citalopram produced a mixed anxiogenic/anxiolytic-like response in the light/dark test in rats and that paroxetine induced an anxiogenic-like response at low doses. The mechanism of action of SSRIs in anxiety is as yet not understood, but because many of these compounds possess affinity for 5-HT_2 receptors, the various receptor subtypes may be implicated in the anxiolytic properties.

6.7. Effects of acute administration of antipsychotics

The administration of cyamemazine at doses of 0.375 and 0.5 mg/kg significantly decreased the percentage of

Table 4
Effects of antidepressants in the light/dark test in mice (acute administration)

Drugs	Effects	Authors
Paroxetine	+	Hascoët et al., 2000b
Amitriptyline	– –	Costall et al., 1989
Citalopram	– –	Griebel et al., 1994
Imipramine	+	Young and Johnson, 1991a,b; Shimada et al., 1995; Bourin et al., 1996
	0	Onaivi and Martin, 1989
Clorgyline	0	Crawley, 1981
Dothiepin	+	Bourin et al., 1996
Mianserin	0	Bourin et al., 1996; Griebel, 1995
Fluoxetine	0	Bourin et al., 1996
Maprotoline	0	Bourin et al., 1996
Viloxazine	0	Bourin et al., 1996
Moclobemide	+	De Angelis and Furlan, 2000
Selegiline	0	De Angelis and Furlan, 2000

"+" anxiolytic-like effects; "–" anxiogenic-like effects; "0" no effect.

Table 5
Effects of antipsychotics in the light/dark test in mice (acute administration)

Drugs	Effects	Authors
Clozapine	+	Bourin et al., unpublished data
Cyamemazine	+	Bourin et al., 2001, 'acute but not repeated doses'
Risperidone	0	Bourin et al., unpublished data

"+" anxiolytic-like effects; "0" no effect.

time spent in the dark compartment (Bourin et al., 2001) (Table 5). However, the dose of 0.5 mg/kg reduced the movements in the light compartment, the number of transitions between the compartments and the latency time to leave the lit compartment. It also dramatically decreased the movements in the dark compartment, but not in a significant manner. Clozapine administration decreased the percentage of time spent in the dark compartment at the dose of 0.015 mg/kg.

The acute administration of two antipsychotics, cyamemazine and clozapine, showed a clear anxiolytic-like effect in the light/dark paradigm (risperidone failed to do so because of its high sedation potential in mice). Both these compounds have antagonistic effects at the 5-HT_3 receptor level and the light/dark test is extremely reliable in detecting positive effects of 5-HT_3 receptor antagonists (Costall and Naylor, 1992; File et al., 1996; Olivier et al., 2000).

6.8. Effects of acute administration of psychostimulants

In a study by Hascoët and Bourin (1998) (Table 6), amphetamine was found to be a stimulant and anxiogenic, producing a significant dramatic increase in the time spent in the dark area, in accordance with the results of Pellow et al. (1985), who found an anxiogenic action in rats in the elevated plus maze. On the other hand, Young and Johnson (1991a,b) demonstrated that amphetamine did not significantly change the time spent in the dark area in the light/dark test.

6.9. Effects of acute administration of neuropeptide receptor ligands

Cholecystokinin-2 receptor antagonists are a novel group of agents with a potential for use in the treatment of anxiety (Griebel et al., 1997a). Experiments with cholecystokinin-2 receptor agonists, such as cholecystokinin-4, have shown them to possess anxiogenic-like effects (Van Megen et al., 1996; Bourin, 1998) (Table 7).

Highly specific and potent antagonists for cholecystokinin-2 receptors have been developed and were found to abolish the anxiogenic-like effects of cholecystokinin-2 receptor stimulation in rodents (Griebel et al., 1997a). The selective cholecystokinin-2 receptor antagonists PD 134308 and PD 135138 were found to be as effective as diazepam in inhibiting aversive responding in the absence of sedation, muscle relaxation or withdrawal phenomenon (Costall et al., 1991; Belzung et al., 1994). Yet none of these compounds were found to be anxiolytic in human (Bourin, 1998; Bourin et al., 1998).

Corticotrophin-releasing factor is a neuropeptide that plays a prominent role in the endocrine, autonomic, behavioural and immune responses to stress, through its action on the major physiological regulator of the hypothalamic–pituitary–adrenal axis (Arborelius et al., 1999). Treatment with antagonists that selectively block CRF_1 receptor action was shown to promote anxiolytic responses in the mouse light/dark box test (Griebel et al., 1998).

The non-peptide tachykinin NK_1 receptor antagonists RP 67580 and (2*S*,3*S*)-CP-96,345, the NK_1 receptor-selective enantiomer of the racemic compound, were tested in Swiss albino mice in the black-and-white box behavioural paradigm. Both qualitatively and quantitatively, (2*S*,3*S*)-CP-

Table 6
Effects of psychostimulant drugs in the light/dark test in mice

Drugs	Effects	Authors
Adrafinil	0	Hascoët and Bourin, 1998
Amineptine	0	Hascoët and Bourin, 1998
Caffeine	0	Hascoët and Bourin, 1998; Shimada et al., 1995
	– –	Imaizumi et al., 1994a,b
Amphetamine	– –	Hascoët and Bourin, 1998; Onaivi and Martin, 1989

" – " anxiogenic-like effects; "0" no effect.

Table 7
Effects of neuropeptides receptors ligands in the light/dark test in mice

Drugs	Effects	Authors
CCK_2 receptor agonist		
CCK 4	0, –	Hascoët and Bourin, 1998; Fink et al., 1994
BC 197	–	Daugé and Roques, 1995
CCK8s	–	Fink et al., 1994
CCK_2 receptor antagonists		
PD 134308	+	Belzung et al., 1994
PD 135138	+	Costall et al., 1991
CI-988 (L-365,260)	+	Hughes et al., 1990; Singh et al., 1991; Costall and Naylor, 1997
CRF_1 receptor antagonists		
CP-154,526	+	Griebel et al., 1998
SSR125543A	0	Griebel et al., 2002
Antalarmin	0	Griebel et al., 2002
NK_1 receptor antagonists		
CP-96,345	– –	Zernig et al., 1993
RP 67580	0	Zernig et al., 1993
Opioid receptor ligands		
Sigma2 ligand Lu 28-179	+	Sanchez et al., 1997

"+" anxiolytic-like effects; " – " anxiogenic-like effects; "0" no effect.

96,345 produced the same behavioural effects as the racemic compound. In contrast, RP 67580 decreased exploratory behavior only in the white section, whereas crossings and rearings in the black section were not changed. In addition, RP 67580 decreased transitions. While the observed changes induced by CP-96,345 are caused by sedation and motor impairment, the effects of RP 67580 may be due to sedation plus an additional anxiogenic effect (Zernig et al., 1993).

The anxiolytic potential of the selective sigma 2 ligand 1-[4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(^{3}H),4-piperidine] (Lu 28-179) was assessed in various animal models of anxiety in rodents. Lu 28-179 facilitated the exploratory behaviour of mice in the black and white two-compartment box over a large dose range (Sanchez et al., 1997).

7. Conclusion

In conclusion, the light/dark test may be useful to predict the anxiolytic-like or anxiogenic-like activity of drug in mice. It has the advantages of being quick and easy to use, without the prior training of animals, food and water deprivation is unnecessary and natural stimuli are used. Transitions have been reported to be an index of activity-exploration because of habituation over time, and the time spent in each compartment to be a reflection of aversion (Belzung et al., 1987), but the best measures seem to be the percentage of time spent and the movements/exploratory behaviour in each compartment (Hascoët and Bourin, 1998).

It has been suggested that some animal models based on spontaneous behaviour or ethologically based models (Lister, 1990) (like the light/dark test) may be more sensitive to the behavioural responses than conditioned paradigms (Griebel, 1996).

The light/dark transition test is limited by its ability to yield false-positive results if of a drug increases general activity. As with many experimental protocols, drugs that affect general motor function will affect light/dark performance. Preliminary screening of locomotor activity (such as an open field or an actimeter test) appears to be necessary and sufficient for eliminating false-positive result. Another problem arises if the white side is insufficiently aversive; the most important feature being the differential between the two sides. Different measures and procedures have been used by different laboratories, so that the effects cannot be reproduced within and between laboratories and this may well have contributed to a number of false-positive results. Different results question the validity of some pre-clinical results and also suggest that more attention must be paid to the publication of nonsignificant effects.

It remains difficult to estimate the value of the test in terms of effect size compared to that of other tests, which predict efficacy in human anxiety disorders (Bourin, 1997).

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