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Limitations in animal model for anxiety disorders: A scientific analysis

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Abstract
Developing an animal model for thought-based disorders like anxiety is an uphill battle in scientific community. More than the face and construct validity, the predictive validity is heavily relied to validate the animal model for anxiety disorders in rats. Among the various conditioned and unconditioned animal models for anxiety disorders, ethological based models like predator encounter is reported to have high validity and reliability because the defensive reactions against predator is largely instinct. For these instinct behaviours to express certain key environmental needs coinciding with their ethology should be provided during the rat developmental stages and their requirement for wellbeing should be studied from their wild conspecifics. But as the architect of the animal house being humans, they mostly extend their comfort to the animals. As a result, the conventional designed cages have provisions of enhanced day light availability, ambient temperature regulation with air coolers normally present in animal house. Additional drawback for the development of animal model for anxiety is the maze design itself. The standard procedure involves studying the rat behaviour in a fixed maze of a particular geometric design. Most mazes design constraint the rat ethology and only explore to what extend the animal is accepting the things normally to be avoided e.g., light chamber entry in the light dark box and open space entry in the elevated plus or zero maze. This review presently discourses the importance of these limitations and the way it will influence the development of animal model for anxiety disorders.

Keywords: Animal model, rats, anxiety disorder, ethology

1. Introduction

Anxiety animal research is a centenary old topic. The laboratory rodents mostly albinos were invariably used as an animal model for anxiety disorders. The idea of developing a close representation of anxiety of humans in animals is an expectation but in reality, it is vain. Because, in Humans anxiety appears to be psycho physiologically based (Edwards *et al.*, 1996) [10] but very rarely appears in overt behaviour (Handley and McBlane, 1993) [13]. Such a profound lack of direct psychiatric observation of behaviour during diagnostic and/or assessment interviews, results in methodological and conceptual difficulties in the integration of human and animal data with respect to drug effects on behaviour (Troisi, 1999) [26]. The metacognition process (Bacow *et al.*, 2010) and mental time travel (Mendl and Paul, 2008) plays a pivotal role in anxiety disorders of humans, but the animal's capacity of these mental attributes is yet to be established (Mendl and Paul, 2008), so it can be presumed to be represented primarily as fear. The crux of the issue lies in the notion that not knowing how an animal feels or what it might be experiencing mentally necessitates inferences with respect to observable behavioural drug effects, but psychometric assessment is primarily relied on to evaluate drug effects on human behavior (Troisi, 1999) [26].

Methodological difference between human and animal studies largely impedes the development of valid animal models of anxiety. Diagnosis then, is not the issue, but whether analogous behavioural symptoms of anxiety between humans and animals, when provoked, can be similarly addressed with anxiolytic drug treatment, is of considerable importance (Troisi, 1999) [26]. This paper addresses the various issues that interfere with normal behavioural expression of rat and how this will influence the reliability of anxiety animal model.

2. Housing conditions

2.1 Behavioural Analysis

The animal behaviour is influenced by both instinct and environmental conditions (Breed & Sanchez, 2010) [4].

The study of ethological responses to different forms of external threats is a logical extension and simulation, in laboratory conditions, of what occurs in nature (innate fear/avoidance). These models are proposed to have a high ethological validity, permitting a more detailed characterization of the behavioural changes induced by the tests (Cole and Rodgers, 1999). In rats, exposure to a live cat or to its odor elicits specific behaviours, such as fight, freezing, risk assessment, and autonomic activation. Defensive behaviours are observed in all mammalian species and occur in response to threatening cues, such as the presence of live predators and environmental hazards (Blanchard & Blanchard, 1989; Blanchard *et al*., 1989) [2]. Live cat exposure is usually resistant to habituation, has a strong contextual conditioning component, and induces anxiogenic-like effects in animals. Therefore, exposure to an ethological stimulus evokes defensive responses that resemble emotional states related to fear and anxiety (Blanchard & Blanchard, 1988; Hendrie *et al.,* 1996) [9, 15].

The basic ground for predator-based models is the set of behavioural responses induced by exposure to a predator, which simultaneously evokes fear and vigilance, creating a typical defensive and risk assessment behaviours. The preparation of animal for behavioural research begins when the rat is pregnant and all way the down till developing in to an adult. The absence of certain "key" stimuli in the physical environment of captive animals can result in a failure to express certain behavioural patterns (Price, 1999) [23], which might be useful in ascertaining in the anxiety-like behaviour. In wild, rat digs burrows and this burrows acts as a shelter and this shelter making is the basic step to protect them from predators, harsh environmental vagaries and to store food. So, the laboratory reared domesticated rats in the early life should be provided with the bedding material that encourage the burrow formation. Nikoletseas and Lore (1981) [20] found that domesticated Norway rats reared in cages with burrows were more aggressive toward strange intruders than rats reared in standard laboratory cages without shelter. Such enhanced defensive reactions are overt and even minor variations caused by pharmacological agents are easily observable.

2.2 Design of laboratory housing apparatus

The design of laboratory rat cages should simulate the features of the natural burrows. The standard laboratory cages are covered with removable grid in the top. This allows enough ventilation and easy inspection for researchers without opening the tray. On contrary, in field, the burrows always open up inside and goes down at an angle and always the top is layered with soil. Rat being prey animals, have to protect themselves from the predators and their location of eye in side with a binocular overlap of 78° (Heffner & Heffner, 1992) [14] hampers the top view. So, their burrows are concealed at the top, which in turn gives a sense of secure feel (Flannelly *et al.,* 1986) [12]. But, in the standard laboratory cages, the adult rat cannot even rear, if it wants to assess the risk in the environment. Such limitations in standard cages may interfere with the key defense behaviour.

The top opening in the standard cages allows the unrestricted light availability, whereas, almost all burrows are dark down inside. Compared with the wild rodents, the albinos' lack the melanin pigment in their pupil of eye and therefore cannot control the light entry into retina of the eye and so most of the rat when exposed to higher light intensities in the cages eventually becomes blind (Burn, 2008) [6]. So, the design of the standard cages should consider the limitations in the laboratory rodents and should have provisions to evade for the environmental vagaries. In our laboratory, the cages are of 30 cm in height, concealed at top and silted side wards. The minor slit prevents the direct light reaching the rat, but let ventilation. The cages are filled with bedding material up to half and the rat constructed burrows and stay inside during the day times.

2.3 Lighting or Light intensity

The intensity of light in the animal house and experimental room should also coincide with the ethology. Rat is crepuscular animals (Calhoun, 1963) [7]. In wild, the rat actively engages in the foraging at dawn and dusk periods of the day. So, the timing of the behavioural experiments should be coinciding with the active hours of the rat i.e., dawn and dusk period. The light inside the animal room should be identical to the light level of burrows. The light level of the experimental room should be identical to the dawn and dusk light level. The light level is an important factor in the navigation of the rat and therefore presence of ambient light level is essential to obtain optimum behavioural results. The bright colored cloth are quite repulsive, therefore should not be worn by the researchers while handling the animals and some laboratories prefer to use red light in the animal room (as rodents visions is not impaired by upper wavelengths, (Burn, 2008)) [6] but nevertheless it is good to provide light matching with their ethology for the reliable data.

2.4 Temperature

Environmental temperature is another concern. In wild conditions, animals can adapt to the temperature by plugging their burrows (Nowak, 1991) [22] and also by regulation of their food intake but in laboratory conditions mostly commercial rat feed are provided *ad libitum* and the temperature is maintained with air-conditioners, coolers and blowers. These machineries create ultrasound sound frequencies which might be inaudible to man, but highly disturbing the rat, as they can able to hear these sound frequencies. Similar disturbance was also created by computers and other human interface (Kelly & Masterton, 1977; Sales *et al*., 1988) [16, 24].

For wild rats, humans itself pose the predatory risk. Owing to domestication, tameness and offering of food, the laboratory rodents behave docile with acquainted human. However, they are quite sensible and responds to new personnel (Price, 1999) [23]. Moreover, the rodents have powerful olfaction (Burn, 2008) [6]. The smell associated with each person is a unique identification mark (Mc Call *et al*., 1969), and any new smell or the change of usual smell like application of deodorant, perfumes, and odorant soaps will provoke the necessary defense reaction (Komori *et al.,* 2003) [17], which might alter the experimental behavioural data and therefore sudden change of animal handlers (Ferreira & Hansen, 1986) [11] or strong odorous substance is not advisable during the ongoing experimentation.

3. Role of automated software packages

Earlier days, manual presence of the researcher in vicinity of behavioural experiments was necessary to note the behaviour. The problem with manual observation is the presence of human pose a predatory risk to animals and the ensuing behaviour results after taking consideration of human factor (either in a positive or negative connotation). Researchers interference obscures the natural behavioural expression. Also, the researcher needs bright light to note minor behavioural changes in the rodents but rodents are aversive to the bright lit situations (Burn, 2008) [6] and on contrary, the rats are comfortable to navigate in dim light, which is disadvantageous to the researcher and nocturnal behavioural experiments cannot be studied manually. Moreover, the researchers fatigue and re-studying and the researcher bias are the further limitations of the manual observations involving behavioural experiments.

The advent of automated tracking software like Any-maze, Ethovision and IR vision analog cameras resolves most of the glitches in manual presence and even enables the tracking of the animals in zero lux light. The automated software enable the researcher to control the experiments by staying far and recording of video footages helps to re-analyses at later date and overcomes the bias and thus increases the reliability of the experimental results (Noldus, 2009). The flip side seems to be cost, and the position of camera being fixed (either side wards view or from the above view) restricts the analysis of animals behavioural expression of the other side, which can be solved by fixing additional cameras to cover the missed positions and cautious faith should be applied to the locomotion tracking data and the interpretation has to be drawn from the contextual animal behaviour.

4. Maze limitations

Light dark box, Elevated plus maze (EPM) and elevated zero maze (EZM) and Open field are the common mazes used to study the anxiety in animals. In EPM or EZM, the animal will be presumed to be in anxiety / fear if it remains in the closed compartment (Walf and Frye, 2007; Shepherd *et al*., 1994) [27, 25]. These apparatuses harness the approach / avoidance conflict. The rats by instinct are inquisitive to explore the new environment (by approach). In EPM or EZM, when a rat takes a risk, and explores naïve elevated open space (by approach and overcoming the avoidance), this grandiose movement actually risks the life of the rat by exposing them to naïve elevated open space. Unlike *Rattus rattus*, commonly called roof rats, *Rattus norvegicus* is a ground dwelling rodent and when a ground dwelling wistar rat, if placed in high ground, which is not a part of ethology habitat, they tend to avoid the freewill locomotion in elevated open space and mostly it is protective and ensures its survival. The classical benzodiazepine, diazepam, used as standard drug for anxiolytic activity, invariably acts to relax the muscle and also hamper the decision-making ability (Lujungberg, 1987) [18]. In such analogous case, the rat movement in the naïve elevated open space in nature cannot be considered resulting from anxiolysis, as this step may even endanger its life either by predisposing to predatory attack or by accidental fall from the elevated floor. Likewise, rat movement in the exposed area in nature, as in open field apparatus (Walsh and Cummins, 1976) [28], also pose a similar life threat from aerial and ground predators. Although, the Light/Dark box have enclosed chambers in both side; the bright light is repulsive to the albinos as they can't handle over 65 lux (Burn, 2008) [6]. So, in the Light dark box, the bright light aversion causes the rat to stay in dark box (Bourin and Hascoët, 2003) [5], but if the rat visits the bright lit area under the anxiolytic, then their vision might be affected (aversion) and their survival will at jeopardy and this befit a question, how a drug making the rats to accept these aversions can be considered as anxiolytic? and why the conventional maze always explore the features far away from their ethology?

5. Discussion and Way Forward

Statistical average is considered as high weight for accepting the experimental data in the behavioural research a typical behaviour from even a single rat if relevant to the context and coinciding with wild ethology, it should be given due importance. To increase the reliability in data, standard comparisons should be drawn from the wild counterparts.

In conclusion, knowledge and incorporation of species-specific perception is necessary to increase the reliability of behavioural data, also the maze should provide a suitable platform for the natural behavioural expression and the effect of various anxiolytic drugs should be studied from both contextual and ethological perspective.

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