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A review of 25 years of the social interaction test

Sandra E. File, Pallab Seth*

Psychopharmacology Research Unit, Centre for Neuroscience, King's College London, Guy's Campus, London SE1 1UL, UK

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Abstract

The social interaction test of anxiety was developed 25 years ago to provide an ethologically based test that was sensitive to both anxiolytic and anxiogenic effects. It is sensitive to a number of environmental and physiological factors that can affect anxiety. It has detected anxiogenic effects of peptides such as corticotropin-releasing factor (CRF) and adrenocorticotropic hormone (ACTH), and anxiolytic effects of neuropeptide Y and substance P receptor antagonists. It has successfully identified neuropharmacological sites of action of anxiogenic compounds and drug withdrawal. Effects of compounds acting on the γ-aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT) systems have been extensively investigated after both systemic administration and microinjection into specific brain regions. The use of this test has, thus, played a crucial role in unravelling the neural basis of anxiety. It is hoped that in the next 25 years, the test will play a crucial role in determining the genetic basis of anxiety disorders.

Keywords: Anxiety; Social interaction; Neuropeptide; GABA receptor; 5-HT (5-hydroxytryptamine, serotonin); Brain region

1. Background

The social interaction test was developed 25 years ago (File and Hyde, 1978) as the first animal test of anxiety that endeavoured to use ethologically relevant sources of anxiety, and to use a natural form of behaviour as the dependent measure. It, therefore, avoided the use of food or water deprivation and electric shock, and did not require extensive training of the animal. The dependent variable is the time spent by pairs of male rats in social interaction (e.g. sniffing, following or grooming the partner). Because the behaviour of one rat influences that of the other, it is important that the pair of rats is treated as a unit, and only one score for the pair is used. Thus, it is possible to use a total or mean score for the pair, or if only one rat is treated (as is often the case for central drug administration), then only the scores of the treated rat should be used. It is a false inflation of the *n* to use separate scores for each member of the pair, as if they were two independent individuals. An increase in social interaction, without a concomitant increase in motor activity, is indicative of an anxiolytic effect, whereas a specific decrease in social interaction indicates an anxiogenic effect. The test conditions are manipulated to generate different levels of anxiety and both the light level and the familiarity of the test arena are manipulated. Thus, there are four test conditions: low light, familiar arena (LF, generating the lowest level of anxiety); high light, familiar arena and low light, unfamiliar arena (HF and LU, generating moderate levels of anxiety); high light, unfamiliar arena (HU, generating the highest level of anxiety). Social interaction is highest when rats are tested in a familiar arena lit by low light, and it decreases as the test conditions become more aversive or anxiogenic. In general, it is easier to see anxiolytic effects (i.e. increases in interaction) when the rats are tested in conditions, such as HU, in which the untreated levels of social interaction are low. Conversely, it is easiest to see decreases in social interaction (i.e. anxiogenic effects) when rats are tested in conditions, such as LF, in which the untreated levels of social interaction are high. This was the first animal test that was able to detect both increases and decreases in anxiety. This opened the way to investigating anxiogenic compounds, and provided a new approach to the neurobiological mechanisms underlying anxiety disorders. From the beginning, attempts were made to validate this test behaviourally and physiologically, as well as pharmacologically, and it has proved sensitive to changes in anxiety generated by nonpharmacological means.

* Corresponding author. Tel.: +44-207-848-6668; fax: +44-207-848-6660.
E-mail address: pallab.seth@kcl.ac.uk (P. Seth).

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2. Behavioural and physiological measures

2.1. Behavioural correlates

In the original validation of this test (File and Hyde, 1978), it was shown that the reduction in anxiety that resulted from a brightly lit or unfamiliar arena was not mediated by olfactory cues (anosmic rats showed the same pattern of decreases in social interaction across the four test conditions). It was also shown that reduced social interaction was not the result of the rats spending more time in other activities, such as walking round or exploring the unfamiliar test arena. In fact, measures of locomotor activity, rearing and exploration showed the same pattern of decrease across the four test conditions as did social interaction, but were less sensitive to the changes. With the decrease in social interaction, there was an increase in other behavioural indices of emotionality, such as freezing, self-grooming and defecation.

In the social interaction test, the rats are normally singly housed for a short period before the test. This is because social isolation reliably increases the time spent in social interaction. As originally standardised, the test used 5 days of individual housing, and Niesink and van Ree (1982) showed that the interaction was maximal after 4–7 days of individual housing. However, it is important to realise that social isolation may also change the response to drugs and, for example, it has been reported to modify the effects of diazepam, chlordiazepoxide and nicotine in the social interaction test (Wongwitdecha and Marsden, 1996; Vale and Montgomery, 1997; Cheeta et al., 2001a). When rats are group-housed and tested, sequential removal of the rats from the cage (cohort removal) may act as a rapid and potent stressor for the remaining rats. When rats were treated in triads, those removed last position had shorter social interaction time and higher body temperature than those removed first (Kask et al., 2001a). However, in rats treated in groups of five, Cheeta et al. (2001a) did not find any difference in the time spent in social interaction or in locomotor scores between the rats removed first from each cage and those removed last.

The test was validated for male, adult rats, and there are some important sex differences in that female rats do not increase social interaction as markedly in response to increasing familiarity with the test arena (Johnston and File, 1991). It is, therefore, possible that social interaction serves a different function in male and female rats and caution should be exercised when interpreting results in females. However, the test has been used in female rats, and Kellogg et al. (1991) have shown that adult female rats exposed in utero to diazepam (2.5 mg/kg) demonstrated a significant effect of the novel environment on social interaction, thus, responding like unmanipulated adult male rats. Adult female rats displayed a pattern of environment-related social interaction similar to that in male rats castrated as juveniles and tested at 60 days. The results from this study indicate that pubertal secretions of gonadal androgen(s) are necessary for the development of environment-related social interaction in adult male rats (Primus and Kellogg, 1990a). Furthermore, while castration of rats as juveniles (day 19) altered the effect of diazepam on social interaction in adult rats, testosterone replacement during puberty (days 30–60) reinstated the ability of diazepam to modify social interaction. The results of these studies demonstrate that gonadal function during puberty is necessary for the development of specific neural systems underlying social interaction in the adult rats (Primus and Kellogg, 1990b). Adolescent male and female rats show the same levels of social interaction and both respond in the same way to manipulations of the test arena (File and Tucker, 1984a). The test can, therefore, be used in both sexes at this age, and it has been shown that there are important sex differences in response to nicotine, with female adolescents being more sensitive to the anxiolytic effects (Cheeta et al., 2001c). There have been no studies of social memory in adolescent rats, but both short-term (15 min) and long-term (24 h) memory for the identity of a conspecific remains unchanged from 5 months of age until 19–27 months (Taylor et al., 1999).

2.2. Environmental factors

Environmental factors have been shown to change social interaction. Thus, rats housed under noisy conditions for 24 days had significantly lower levels of social interaction than rats housed under quiet conditions (File, 1994). Rats exposed for 5 min to the odour of a cat or fresh rat blood showed significant anxiogenic effects when later tested in the social interaction test (Zangrossi and File, 1992a), but these effects were reversed by treatment with chlordiazepoxide (Zangrossi and File, 1992b). Somewhat surprisingly, previous restraint stress did not change social interaction (Chaouloff et al., 1994), although prenatal exposure to a mild chronic variable stress did decrease social behaviour in rat pups tested at day 42 (Cabrera et al., 1999). Early experience can also change the levels of social interaction. Rats that experienced 8 h of maternal separation every other day between postnatal days 2 and 10 had significantly lower levels of social interaction and enhanced stress-induced plasma adrenocorticotropic hormone (ACTH) concentrations than nonseparated controls (Maciag et al., 2002).

Food deprivation (to 85% free feeding weight) is without effect on social interaction (Genn et al., submitted for publication), but the soya content of rat diet does have profound effects. Because soya is a cheap source of protein, most rat diet contains high levels of soya isoflavones (around 150 mg/kg). This diet is sufficient to significantly reduce social interaction and increase the plasma corticosterone response to this test when compared to the responses of rats fed a soya-free diet (Hartley et al., in press).

2.3. Plasma ACTH and corticosterone

Exposure to the social interaction test increases plasma corticosterone concentrations (e.g. File, 1988), and these are increased across the test conditions LF, HF, LU and HU (File and Peet, 1980). Plasma concentrations of ACTH were also significantly increased after testing in the high light compared with the low light condition (File, 1984). Thus, exposure to the social interaction test changes stress hormones as well as generating behavioural changes indicative of anxiety.

Rats from the same outbred strain (hooded Lister), but from different suppliers, had striking differences in social interaction, and those with the lowest level of social interaction (indicating higher levels of anxiety) had higher levels of defecation and higher concentrations of corticosterone (File and Vellucci, 1979). Fawn-hooded rats have a higher baseline level of corticosterone and an increased freezing response to stress and markedly lower levels of social interaction than Wistar or Sprague–Dawley strains when tested in the HU and LF test conditions (Kantor et al., 2000).

However, endogenous administration of corticosterone has an anxiolytic effect (File et al., 1979c), either due to opposing central nervous system (CNS) effects or to feedback inhibition of ACTH release. It is interesting that although brief exposure (1.5 min) to the social interaction test enhanced defensive burying (indicating an anxiogenic effect), a longer exposure to the test (15 min) inhibited defensive burying, indicating an anxiolytic effect (Saldivar-Gonzalez et al., 2000). Brief exposure to the social interaction test increases ACTH concentrations, whereas after 15 min, the corticosterone concentrations would have increased, and it is possibly these that were contributing to the anxiolytic effect. Adrenalectomised rats had very low levels of social interaction, but those given replacement corticosterone did not differ from sham-operated controls (File et al., 1979c).

2.4. Brain 5-hydroxytryptamine (5-HT)

There is evidence that exposure to the high light conditions of the social interaction test increases 5-HT activity, particularly in limbic areas. There were increases in K^+-evoked [^{3}H]5-HT release from hippocampal, but not cortical, slices taken from rats exposed to the HF test conditions (File et al., 1993c). Using in vivo microdialysis, increases in 5-hydroxyindoleacetic acid (5-HIAA), the chief metabolite of 5-HT, were found in the hippocampus, amygdala and entorhinal cortex, but no changes in the frontal or temporal cortex after exposure to the HU test condition (Ge et al., 1997). The changes in 5-HT function were also accompanied by changes in K^+-evoked γ-aminobutyric acid (GABA) release from hippocampal slices and changes in GABA uptake in cortical slices from rats exposed to the HF test condition (File et al., 1993c) and by increased levels of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) in the amygdala of rats exposed to the HU test condition (Ge et al., 1997).

The Fawn-hooded rats, a strain with genetically impaired 5-HT storage and reuptake system, showed significantly higher anxiety than Sprague–Dawley rats in the social interaction test (Kantor et al., 2000). Interestingly, a line of rats specially selected for differential 5-HT$_{1A}$ receptor function also showed striking differences in the social interaction test (Gonzalez et al., 1998a; File et al., 1999), and the line with very low levels of social interaction had an abnormal response to 5-HT$_{1A}$ receptor ligands when injected into the dorsal hippocampus (Gonzalez et al., 1998a). In contrast, lines of rat selected on the basis of their acquisition of a two-way active avoidance response in a shuttle box did not differ in the social interaction test, suggesting that the type of anxiety measured by this test differs from conditioned fear responses (Kulikov et al., 1995).

3. Social interaction in mice and gerbils

3.1. Mice

de Angelis and File (1979) showed that Swiss albino mice showed the same decrease in social interaction as rats when the light level was manipulated, but that the changes in response to the familiarity of the test arena were less reliable. Lister and Hilakivi (1988) found similar results, using male NIH Swiss strain of mice and, furthermore, drugs that have clear anxiolytic and anxiogenic profiles in the rat test did not have specific effects in the mouse test (Lister and Hilakivi, 1988; Hilakivi et al., 1989). Cutler et al. (1997) failed to find anxiolytic effects of buspirone, ondansetron or tianeptine using social interactions in mice as the dependent measure.

Krsiak et al. (1984) and Krsiak and Sulcova (1990) measured social interaction between isolated and group-housed mice, with only the former receiving drug treatment. In general, drugs that are effective anxiolytics in man increased social interaction in these mice, but it was often necessary to select subgroups of ‘timid’ or ‘aggressive’ mice in order to see effects. Olivier and Mos (1988) and Olivier et al. (1989) used isolation-induced aggression to extensively investigate the role of the 5-HT system in aggressive behaviour. However, this is a more specialised use of social behaviour, and they concluded that it should be used in conjunction with other tests to fully characterise the effects of serotonergic drugs on social behaviour.

The high rates of social reactivity exhibited by isolated male mice are mediated, at least in part, by an increase in the density or an increased sensitivity of D_1 dopamine receptors (Lewis et al., 1994; Gariepy et al., 1995). These behavioural effects of isolation are reversible, and that changes in dopaminergic function support this reversibility (Gariepy

et al., 1998). Recent studies pointed out that the D_3 dopamine receptor could also play a role in this type of emotional behaviour (Rodriguez-Arias et al., 1999).

In many mutant mice, such as knock-outs of the 5-HT_{1B} receptor (Sandou et al., 1994) and pre-proenkephalin (Koenig et al., 1996), aggressive behaviours are increased. However, in mice lacking gastrin-releasing peptide receptor, the nonaggressive social responses in social interaction tests are significantly increased, whereas aggressive social responses are unchanged (Yamada et al., 2000). The multisynaptic neural pathways linking the chemosensory systems of the olfactory bulbs with the rostral hypothalamus may be involved in the regulation of intermale aggression in mice (Edwards et al., 1993).

3.2. Gerbils

Most recently, a social interaction test has been developed in gerbils. This test has been less extensively validated, and there are a number of factors that differ for this species. We found that it was not necessary to socially isolate the gerbils before the test and, indeed, more reliable data were obtained when the animals were group-housed. We also found that the HF test condition was the most sensitive to anxiolytic effects and was also sensitive to anxiogenic effects. Using this test, diazepam, nicotine, 8-hydroxy-2-di-*n*-(propylamino)tetralin (8-OH-DPAT) and a substance P receptor antagonist were found to have anxiolytic effects (increased social interaction), whereas an acute dose of fluoxetine had an anxiogenic effect (Cheeta et al., 2001e; File et al., 2001).

4. Effects of neuropeptides

4.1. Corticotropin-releasing factor (CRF)

Because the social interaction test was not developed solely to detect the effects of the benzodiazepines, it is not surprising that it has proved to be sensitive to the effects of a wide range of drugs and neuropeptides. Intracerebroventricular injection of corticotropin-releasing hormone has an anxiogenic effect (Dunn and File, 1987), and this could be reversed by chronic, but not acute, treatment with fluoxetine (To et al., 1999). Combined infusions of CRF and arginine vasopressin into either the lateral ventricle or the amygdala have synergistic effects on aggressive, investigative and other behaviours occurring during social interaction between male rats (Elkabir et al., 1990). Urocortin, a peptide with affinity for both CRF_1 and CRF_2 receptors, when injected into the basolateral nucleus of the amygdala, produced an anxiogenic response. After repeated injections of subthreshold doses, it produced a 'priming' effect that results in a panic-like reaction (Sajdyk et al., 1999a). A nonpeptidergic CRF_1 receptor antagonist, DMP696, had anxiolytic effects in both previously maternally separated rats and in their controls (Maciag et al., 2002), which suggests that there may be a tonic activation at the CRF receptors. Like DMP696, the nonpeptidergic CRF_1 receptor antagonist, CP154,526 had an anxiolytic action which was independent of monoamine release (Millan et al., 2001). Astressin, a CRF receptor antagonist, though ineffective per se, reversed the anxiogenic effects of urocortin when administered into the basolateral amygdala (Sajdyk and Gehlert, 2000). However, another CRF_1 receptor antagonist, CRA 1000, had no major antianxiety effect in nonstressed animals (Harro et al., 2001).

4.2. ACTH

Both systemic and intracerebroventricular administration of ACTH-(1-24) and ACTH-(4-10) had anxiogenic effects (File and Vellucci, 1978; File, 1979a; File and Clarke, 1980). α-melanocyte-stimulating hormone (α-MSH) also had an anxiogenic effect, but the trisubstituted synthetic analogue of ACTH-(4-9), Org 2766, increased social interaction, but only when the rats were tested in familiar arenas (File, 1981). As Org 2766 was without effect in unfamiliar test arenas, it would not seem correct to interpret this effect as being an anxiolytic action. The septum (in the region of the lateral and fimbrial nuclei) has been identified as one brain region important for mediating the effects of ACTH in this test (Clarke and File, 1983), as has the dorsal raphe nucleus (File et al., 1979b). However, the median raphe nucleus does not seem to play a role (File et al., 1979b), and the infundibulum can be excluded as an important site (Clarke and File, 1981).

4.3. Cholecystokinin (CCK), substance P and neuropeptide Y

Intracerebroventricular administration of cholecystokinin octapeptide (CCK-8) had an anxiogenic effect that was fully reversed by chronic treatment with fluoxetine and partially reversed by chronic treatment with ipsapirone (To and Bagdy, 1999). Nonpeptide CCK_2 receptor antagonists (Costall et al., 1991) and substance P (NK_1) receptor antagonists (File, 1997, 2000; Vassout et al., 2000) have been reported to have anxiolytic effects in the rat social interaction test, and the latter have also been found to be effective in gerbil social interaction tests (Cutler, 1994; Cheeta et al., 2001e). After unilateral microinjection into the nucleus basalis magnocellularis region, substance P itself was found to exert anxiolytic-like effects in the rat elevated plus-maze and social interaction test (Hasenohrl et al., 1998).

Neuropeptide Y was without effect when administered into the central nucleus of the amygdala, but had an anxiolytic effect when infused into the basolateral nucleus of the amygdala. This effect was antagonised by co-administration of a specific neuropeptide Y Y_1 receptor antagonist (Sajdyk et al., 1999b). In contrast to the anxiolytic effect mediated by neuropeptide Y Y_1 receptors in

this region, neuropeptide Y Y_2 receptors seem to mediate anxiogenic effects because injection of a neuropeptide Y Y_2 receptor agonist, C2-NPY, into this region had an anxiogenic effect that was reversed by alprazolam (Sajdyk et al., 2002).

Bilateral microinjections of neuropeptide Y into the dorsocaudal lateral septum (but not into the intramedial septum) dose-dependently decreased anxiety in the social interaction test of rats. Microinjection of the neuropeptide Y receptor-selective antagonists in the dorsocaudal lateral septum revealed that neither neuropeptide Y Y_1 receptor nor neuropeptide Y Y_2 receptor selective antagonists had effects on experimental anxiety on their own, suggesting that there is no tonic mediated of an anxiolytic effect in this region (Kask et al., 2001b). However, the neuropeptide Y receptors in the dorsal periaqueductal grey seem to be exerting a tonic anxiolytic effect, since administration of neuropeptide Y Y_1 receptor antagonists into this region had anxiogenic effects (Kask et al., 1998).

5. Anxiogenic drugs

5.1. Benzodiazepine receptor antagonists and inverse agonists

Although agonists at the benzodiazepine receptor have anxiolytic effects (see Section 7), the social interaction test was the first to show that some compounds acting at this receptor had opposite effects. The benzodiazepine receptor antagonist, flumazenil, had an anxiogenic action (File et al., 1982) that could be reversed by subchronic administration of chlordiazepoxide (File and Pellow, 1984). Another benzodiazepine receptor antagonist, ZK 93426, also had an anxiogenic action (File et al., 1986). Other β-carbolines, such as ethyl-β-carboline-3-carboxylate (β-CCE), propyl-β-carboline-3-carboxylate (β-CCP) and FG 7142, which came to be known as inverse agonists, had anxiogenic effects that could be reversed by either chlordiazepoxide or by the receptor antagonist, flumazenil (File et al., 1982, 1984, 1985; File and Lister, 1983a; File and Pellow, 1984). The anxiogenic actions of FG 7142, could be antagonised by triazolobenzodiazepines (File and Pellow, 1985d) and by a silent dose of sodium phenobarbitone (Johnston and File, 1989). Interestingly, although repeated injections of FG 7142 causes chemical kindling of seizures, kindled animals that were tested undrugged showed no changes in social interaction or in two other tests of anxiety, suggesting that prolonged changes in seizure threshold can occur without apparent changes in anxiety (Taylor et al., 1988). Another possible inverse agonist, the quinoline CGS 8216, also had anxiogenic effects in both adolescent (File and Tucker, 1984b) and in adult rats (File and Lister, 1983a), but these were not antagonised by flumazenil, suggesting that the benzodiazepine receptor may not have been its site of action (File and Lister, 1983a).

5.2. Picrotoxin and pentylenetetrazole

Another site on the $GABA_A$–benzodiazepine receptor complex also mediates anxiogenic drug effects. Picrotoxin and a convulsant benzodiazepine Ro 5-3663, which also acts at the picrotoxin site, have anxiogenic effects (File and Pellow, 1983; File and Lister, 1984). Pentylenetetrazole also had an anxiogenic action, and this was enhanced (rather than reversed) by flumazenil (File and Pellow, 1985a). However, the anxiogenic action of pentylenetetrazole could be antagonised by triazolobenzodiazepines (File and Pellow, 1985d) and by sodium phenobarbitone in a dose that had no intrinsic effects (Johnston and File, 1989).

5.3. The peripheral-type of benzodiazepine receptor

Two compounds that act at the peripheral-type of benzodiazepine receptor, PK 11195 and Ro 5-4864, also had anxiogenic effects in the social interaction test and increased plasma corticosterone concentrations (File and Lister, 1983b; File and Pellow, 1985b,c).

5.4. Yohimbine, caffeine and amphetamine

Yohimbine has anxiogenic effects (Pellow et al., 1985; File and Pellow, 1985d; File, 1986b; Bhattacharya et al., 1997). The anxiogenic action of yohimbine was attenuated in rats that had been treated neonatally with the benzodiazepines, diazepam or lorazepam (File, 1986b), and could be antagonised in adult rats by acute administration of triazolobenzodiazepines (File and Pellow, 1985d). Although yohimbine has weak actions at the benzodiazepine receptor, its anxiogenic action is unlikely to be mediated by this site because neither chlordiazepoxide nor flumazenil could antagonise it, whereas the α_2-adrenoceptor antagonist, clonidine, was able to (Pellow et al., 1985).

Caffeine has anxiogenic effects (File et al., 1988; Baldwin et al., 1989; Baldwin and File, 1989a; Bhattacharya et al., 1997). The anxiogenic effect of caffeine was reversed by chlordiazepoxide, but not by flumazenil, suggesting that it was not mediated by an action at the benzodiazepine receptors and that the reversal by chlordiazepoxide was a functional reversal (Baldwin and File, 1989a). Unexpectedly, caffeine and yohimbine antagonized each others' effects in the social interaction test (Baldwin et al., 1989). After chronic treatment with caffeine (21 days administration in the drinking water), there was tolerance to the anxiogenic effect (File et al., 1988).

Like caffeine, amphetamine decreased the time spent in social interaction, but also increased locomotor activity (File and Hyde, 1979) and, therefore, it is difficult to be sure whether these are two independent effects or whether the decrease in social interaction was the result of response competition. Following systemic administration of amphetamine, rats with medial prefrontal cortex lesions increased locomotor activity but their levels of social interaction did

not decrease (Gonzalez et al., 2000), which supports for two independent effects. In other studies, acute and subchronic administration or continuous infusion of D-amphetamine in rats induced locomotor hyperactivity apart from any consistent effects on social behaviour (Sams-Dodd, 1995, 1998a); active social interaction was only slightly decreased at the lowest dose (Sams-Dodd, 1998a).

5.5. Other anxiogenics

Quinine, a cinchona alkaloid, induced a dose-related anxiogenic activity in rats (Bhattacharya and Mitra, 1992). Single injections of *Ginkgo biloba* extract (EGb 761), given 30 min prior to testing, or repeated oral administration of the extract for 8 days, significantly decreased social contact. Diazepam injected to animals that had received repeated oral treatment of EGb 761 increased social interaction to an extent greater than observed with diazepam alone (Chermat et al., 1997). However, in another study, EGb 761 and Ginkgolic acid conjugates isolated from the leaves of Indian *G. biloba*, though anxiolytic in other models of anxiety were without any significant effect in the social interaction test (Satyan et al., 1998).

Centrally administered scorpion (*Mesobuthus tamulus*) venom induced a dose-related anxiogenic response which was qualitatively comparable to that produced by yohimbine (Bhattacharya, 1995). Crotoxin, the major component of South American rattlesnake (*Crotalus durissus terrificus*) venom, decreased the social interaction time. In contrast to crotoxin, irradiated crotoxin was unable to induce behavioural alterations (Moreira et al., 1997). Both diazepam and flumazenil antagonized the crotoxin-induced behavioural alterations which suggest a role of GABAergic–benzodiazepine system in the crotoxin-induced anxiogenic effect (Moreira et al., 2000).

Streptozotocin-induced diabetic rats exhibited augmented anxiety on various experimental paradigms including the social interaction test, and the anxiolytic effect of diazepam was less marked in diabetic rats (Ramanathan et al., 1998). On the contrary, no significant changes in the time spent in social interaction or aggressive behaviour were found in the streptozotocin-treated mice (Hilakivi-Clarke et al., 1990). Eight-week-old offsprings of streptozotocin-diabetic mothers showed anxiogenic activity in the social interaction test (Ramanathan et al., 2000). The increased anxiety-related behaviours in streptozotocin-induced diabetic rats may be related to impaired corticosterone and hippocampal serotonergic systems (Thorre et al., 1997; Bellush et al., 1991).

Two other drugs, naloxone and L-fenfluramine, reduced social interaction, but also reduced motor activity and, therefore, it cannot be concluded that they have specific anxiogenic effects and it is more likely that they were producing nonspecific decreases in spontaneous behaviours (File, 1980b; File and Guardiola-Lemaitre, 1988). The endogenous neuroactive monoamine, β-phenylethylamine, is also effective in reducing the social interaction in mice and the effect was prevented by diazepam (Lapin, 1990). Phencyclidine (PCP), a hallucinogenic drug that can mimic several aspects of the schizophrenic symptomatology in healthy volunteers, dose-dependently induces stereotyped behaviour and decreased levels of active social interaction in rats and these behaviours can specifically be inhibited by antipsychotic drugs (Sams-Dodd, 1995, 1997, 1998b, 1999).

In the social interaction test, in general, antidepressant drugs have been found to have anxiogenic effects when given acutely, as is also found clinically. The monoamine uptake inhibitor, phenelzine, had anxiogenic effects even after 21 days of administration (Johnston and File, 1988b). In addition, both the tricyclic antidepressant, imipramine, and the selective serotonin uptake inhibitor, fluoxetine, have been found to have anxiogenic effects (Pellow and File, 1987; File et al., 1999; To et al., 1999; To and Bagdy, 1999; Bagdy et al., 2001). The anxiogenic effects of fluoxetine and sertraline were antagonised by a selective 5-HT_{2C} receptor antagonist (Bagdy et al., 2001). The selective 5-HT uptake inhibitor, citalopram, also had an anxiogenic effect that seemed to be mediated by 5-HT_{2C} receptors (Dekeyne et al., 2000b).

The 5-$HT_{2C/2B}$ receptor agonist, *m*-chlorophenylpiperazine (mCPP), also has anxiogenic effects (Kennett et al., 1989; Kantor et al., 2000; Bagdy et al., 2001), which were not influenced either by previous housing (single versus grouped) or by restraint (2 h, 24 h previously) (Kennedy et al., 1993). Evidence indicated that the anxiogenic effects of mCPP might be mediated by activation of 5-HT_{1C} receptors (Kennett et al., 1989; Kennedy et al., 1993).

6. Drug dependence

6.1. Benzodiazepine withdrawal

Whilst the benzodiazepines have clear anxiolytic effects after subchronic treatment (e.g. 5 days), after longer treatment (e.g. 21 days), tolerance develops to this effect (Vellucci and File, 1979), and after even longer treatment (28 days) with a high dose, an anxiogenic effect can be seen (Fernandes et al., 1999). When rats are withdrawn from chronic treatment with benzodiazepines, an anxiogenic response is seen and this can be reversed by administration (in the drug-free state) of the benzodiazepine receptor antagonist, flumazenil (Baldwin and File, 1989b). Furthermore, administration of flumazenil during the course of chronic benzodiazepine treatment can prevent the development of this anxiogenic withdrawal response (Baldwin and File, 1989b; Baldwin et al., 1990). It, therefore, seems that flumazenil is able to restore the benzodiazepine receptor to a drug-naïve state (File and Hitchcott, 1990). The anxiogenic response seen on withdrawal from chronic benzodiazepines can also be reversed by baclofen, the $GABA_B$ receptor agonist (File et al., 1991a), and by the tricyclic antidepressant, tianeptine, which has the unusual property of increas-

ing 5-HT uptake (Andrews and File, 1993; File et al., 1993b). The withdrawal response could also be reversed by a low dose of the 5-HT_{1A} receptor agonist, buspirone (File and Andrews, 1991), and by 5-HT_3 receptor antagonists (Costall et al., 1989; Andrews and File, 1992). These results, together with those found in another test of anxiety, the elevated plus-maze, led to the suggestion that during withdrawal from benzodiazepines, the anxiogenic response resulted from increased 5-HT release in terminal areas (see Andrews and File, 1993; File and Andrews, 1993). This was given further support by the finding that injection of 8-OH-DPAT into the median raphe nucleus reversed the withdrawal response from diazepam (Andrews et al., 1997).

6.2. Ethanol withdrawal

A very similar pattern of results was obtained on withdrawal from chronic ethanol treatment. In alcohol-preferring P rats, termination of alcohol intake after 6 weeks of voluntary alcohol consumption resulted in increased anxiety in both the social interaction and elevated plus-maze tests (Kampov-Polevoy et al., 2000). In other strains of rats given an enforced ethanol diet, there was also a significant anxiogenic effect on withdrawal from ethanol that could be reversed by the benzodiazepine receptor antagonists, flumazenil (File et al., 1989, 1992), by the benzodiazepine, chlordiazepoxide (File et al., 1992), and by the $GABA_B$ receptor agonist, baclofen (File et al., 1991b, 1992). The 5-HT system has also been implicated in mediating this ethanol withdrawal response and the tricyclic antidepressant, tianeptine, which has the unusual profile of increasing 5-HT uptake reversed the anxiogenic response (File et al., 1993a), as did the selective 5-HT_3 receptor antagonist ondansetron (Costall et al., 1990b) and a 5-HT_{2C} receptor antagonist (Knapp et al., 2001). However, although the angiotensin-converting enzyme inhibitor SQ29,852 reversed the anxiogenic response, this was not reversed by captopril (Costall et al., 1990a) or by the calcium channel antagonist, nitrendipine (File et al., 1989, 1991b, 1992). Chronic exposure to noise during the chronic ethanol treatment prevented the ethanol withdrawal anxiety response, suggesting that stress modifies the GABA receptor complex in such a way that the changes mediating dependence do not occur (File, 1994). Repeated cycles of ethanol withdrawal resulted in an enhanced anxiogenic effect in both degree and in duration (Overstreet et al., 2002).

6.3. Nicotine withdrawal

Nicotine can have both anxiolytic and anxiogenic effects in the social interaction test, the direction depending both on the dose and time of administration (File et al., 1998; Irvine et al., 1999). After 7 days of treatment, there is tolerance to the anxiogenic effects (Irvine et al., 1999), and this was shown to be mediated by changes in the 5-HT release in the dorsal hippocampus (Irvine et al., 2001a). Tolerance to the anxiolytic effects was shown to be mediated by the 5-HT_{1A} neurones in the dorsal raphe nucleus (Cheeta et al., 2001b). Seventy-two hours after withdrawal from 7 days of treatment with this low dose of nicotine (0.1 mg/kg/day), there was a significant anxiogenic response and this could be reversed by nicotine injected subcutaneously or directly into the dorsal raphe nucleus. In animals that had been self-administering nicotine (0.45 mg/kg/day) for 4 weeks, it was shown that the daily administration of nicotine infusions still had an anxiogenic effect, but no further changes were seen 24 or 72 h after withdrawal from this regime (Irvine et al., 2001c). When rats were given nicotine for 4 weeks by intravenous injections, subcutaneous injections or infusion by subcutaneous minipumps, there was partial tolerance in all groups to the anxiogenic effects, but somewhat surprisingly, the rate of development of tolerance did not differ for the different treatment regimes. Perhaps most intriguingly of all, after 7 days of nicotine administration, there was cross-tolerance to the anxiolytic effects of the benzodiazepine, midazolam (Irvine et al., 2001b).

7. Anxiolytic candidates

7.1. Benzodiazepines, barbiturates and ethanol

The benzodiazepines are still the most widely used and effective anxiolytic drugs, and many of these have been found to be effective in the social interaction test (see Table 1 for summary).

The social interaction test reflects well the difference between acute and chronic benzodiazepine treatment. With acute administration, the benzodiazepines have their usual sedative effect in all of the test conditions (File et al., 1976; File, 1979b, 1982). In contrast, after 5 days of pretreatment, benzodiazepines no longer produce sedation, and rats maintain a steady level of social interaction regardless of the test conditions (File and Hyde, 1978, 1979; File, 1980a).

Neonatal administration of high doses of benzodiazepines increased social interaction when the pups were tested undrugged in adolescence indicating persisting anxiolytic

Table 1
Lowest effective dose of each benzodiazepine in the social interaction test in rat, mouse and gerbil

Benzodiazepine	Dose (mg/kg)	Species	References
Chlordiazepoxide	5	Rat	File and Hyde, 1978
Diazepam	0.125	Rat	Costall et al., 1992
	1	Mouse	de Angelis and File, 1979
	0.1	Gerbil	File et al., 2001
Flunitrazepam	2	Rat	Guy and Gardner, 1985
Flurazepam	0.5	Rat	File and Hyde, 1979
Lorazepam	0.25	Rat	File, 1980a
Nitrazepam	0.2	Rat	Gardner and Guy, 1984
Desmethyldiazepam	2	Mouse	de Angelis and File, 1979
Oxazepam	10	Rat	Gardner and Guy, 1984
Triazolam	No effect	Rat	File, 1980a

effect (File, 1986a). When the rats were tested undrugged in adulthood, there were no significant effect of neonatal administration of benzodiazepines (File and Tucker, 1983b; File, 1986b). However, neonatal diazepam treatment significantly reduced the anxiogenic effects of yohimbine (File, 1986b).

Prior to the introduction of the benzodiazepines, the barbiturates, which act at a site on the $GABA_A$–benzodiazepine receptor complex, were widely used for the treatment of anxiety. Barbiturates also increase social interaction, and the only difference in their profile from that of the benzodiazepines is that they increase social interaction even in the LF test condition. A similar pattern to that seen with the barbiturates has been seen with neuropeptide Y when administered into the basolateral amygdala (Sajdyk et al., 1999b) and in rats treated neonatally with the CGS 8216 and subsequently tested as adolescents (File and Tucker, 1984b) and adults (File and Tucker, 1984c).

Ethanol also acts at the $GABA_A$–benzodiazepine receptor complex, and in the adult rat, low doses of ethanol were anxiolytic, but higher doses caused motor incoordination (File, 1980a; File et al., 1976). Similarly, in adolescent rats, the effects of ethanol were dose-dependent and biphasic. Low doses of ethanol (0.25–0.75 g/kg) produced apparent social facilitation (increased social activity and enhanced social preference), whereas higher doses (3 and 4 g/kg) caused social inhibition (decreased social activity and avoidance of a peer) in a modified dyad social interaction test (Varlinskaya et al., 2001).

7.2. 5-HT receptor ligands

Ever since the introduction of the benzodiazepines, it has been recognised that the 5-HT system plays a crucial role in anxiety. Following the identification of 5-HT receptor subunits, there has been a major research endeavour to determine the roles of these in mediating anxiety.

7.2.1. 5-HT_3

The anxiolytic effects of selective 5-HT_3 receptor antagonists were controversial in animal anxiety models. Tyers et al. (1987), Jones et al. (1988), Costall et al. (1988, 1989, 1993), Barnes et al. (1990a,b, 1992) and Costall and Naylor (1992) reported anxiolytic effects of 5-HT_3 receptor antagonists in a wide range of animal tests, including the social interaction test. Other groups also reported anxiolytic effects of different 5-HT_3 receptor antagonists in rats (Piper et al., 1988; Dunn et al., 1991; Blackburn et al., 1993; Eguchi et al., 2001) and in gerbils (Cutler, 1990; Cutler and Piper, 1990). However, Johnston and File (1988a) and File and Johnston (1989) were unable to replicate these findings and reported that these drugs were ineffective. These compounds were subsequently found to be ineffective in the clinic (Romach et al., 1998; Schweizer and Rickels, 1991; Wilde and Markham, 1996) and, thus, it seems unlikely that the 5-HT_3 receptor plays a major role in anxiety disorders (also, see the review of Olivier et al., 2000). It remains unclear why some groups reported false positives for these compounds in the social interaction test.

7.2.2. 5-HT_{1A}

Following the discovery that the anxiolytic buspirone had partial agonist/agonist actions at the 5-HT_{1A} receptors, there has been major research activity into this receptor subtype. As has been the case clinically, the anxiolytic action of these compounds has not been universally seen in animal tests, and Table 2 provides a summary of the results. It is thought that the reason that 5-HT_{1A} receptor agonists have anxiolytic effects at low doses and anxiogenic effects at high doses is because the former reflects their actions at presynaptic receptors in the raphe nuclei and the latter is the result of actions at postsynaptic receptors in the terminal areas. This issue is discussed in detail in Section 8.

7.2.3. 5-HT_2

Evidence that 5-HT_2 receptors could mediate responses in the social interaction test came from the effects of several antagonists (ketanserin, ritanserin, MDL11939, methysergide and RP62203), which although were ineffective when administered alone were able to antagonise the inhibitory effects of 5-HT in the rat social interaction test (Costall and Naylor, 1995). Iloperidone, a novel atypical antipsychotic agent currently under clinical development with potent 5-HT_{2A} receptor antagonistic property, increased interaction score in social interaction (Szewczak et al., 1995). The 5-HT_{2C} receptors seem to be of greater importance than the 5-HT_{2A} receptors. The 5-HT receptor agonists, mCPP and 1-[3-(trifluoromethyl)phenyl]piperazine (TFMPP) reduced total interaction time in a rat social interaction test under LF conditions (Kennett et al., 1989). This anxiogenic effect of mCPP on social interaction was blocked by three antagonists which have high affinities for both 5-HT_{2C} and 5-HT_{2A} receptors: mianserin, cyproheptadine and metergoline, but not by ketanserin or ritanserin. Six antagonists with high affinity for 5-HT_{1C} receptors; mianserin, (+) mianserin, 1-naphthyl piperazine, ICI 169 369, pizotifen and LY 53857 all increased the time spent in active social interaction under HU test conditions, indicating an anxiolytic action (Kennett et al., 1989). SB 242084, the first reported selective potent and brain penetrant 5-HT_{2C} receptor antagonist, exhibited an anxiolytic-like profile in the rat social interaction test (Kennett et al., 1997). The finding that the 5-$HT_{2C/2B}$ receptor agonist, mCPP, causes behavioural indications of anxiety in both animal models and humans prompted Kennett's group that selective 5-$HT_{2C/2B}$ receptor antagonists might be useful anxiolytic agents. SB 200646A, SB 206553 and SB-221284, all 5-$HT_{2C/2B}$ receptor antagonists, were found to be anxiolytic in rat social interaction test (Kennett et al., 1994, 1996b; Bromidge et al., 1998).

It is not yet clear whether 5-HT_{2C} receptor agonists are anxiogenic. Ro 60 0175, a 5-HT_{2C} receptor agonist, reduced

Table 2
Drugs acting on the 5-HT_{1A} receptors in the social interaction test in the rat

Drug	Dose	Effect	References
Buspirone	0.25–2.5 mg/kg	0	File, 1984
	5.0–20.0 mg/kg	+	Guy and Gardner, 1985
	40–200 ng CNS	+	Higgins et al., 1988, 1992
	5.0–10.0 mg/kg	+	Dunn et al., 1989
	0.2 mg/kg	+[a]	File and Andrews, 1991, 1994
	0.8 mg/kg	0[a]	File and Andrews, 1991
	0.25 or 0.5 mg/kg	0	Costall et al., 1992
	1 mg/kg	+	Costall et al., 1992
	2 mg/kg	0	Costall et al., 1992
	0.16 mg/kg	+	Dekeyne et al., 2000a
	0.63 mg/kg	+	Dekeyne et al., 2000a
	2.5 mg/kg	–	Dekeyne et al., 2000a
	3 or 10 mg/kg 4 h after injection	+	Haller et al., 2000
	3 or 10 mg/kg 1 h after injection	0	Haller et al., 2000
8-OH-DPAT	20–100 ng CNS	+	Higgins et al., 1988
	0.125–0.25 mg/kg	+	Dunn et al., 1989
	0.02–1 μg CNS	+	Higgins et al., 1992
	50 ng dorsal raphe nucleus	+	Hogg et al., 1994
	50 or 100 ng ventral hippocampus	0	Hogg et al., 1994
	200 ng median raphe nucleus	+	Andrews et al., 1994; File et al., 1996
	50 or 100 ng median raphe nucleus	0	Andrews et al., 1994
	100 ng dorsal hippocampus	–	Andrews et al., 1994; File et al., 1996
	0.25 or 0.5 mg/kg	+	Picazo et al., 1995
	0.125 mg/kg	0	Picazo et al., 1995
	0.1 μg/μl dorsal raphe nucleus	+	Picazo et al., 1995
	0.1 μg/μl intrahippocampal	0	Picazo et al., 1995
	50–200 ng basolateral nucleus of the amygdala complex	–	Gonzalez et al., 1996
	200 or 500 ng lateral septum	–	Cheeta et al., 2000b
	0.04 mg/kg	0	Dekeyne et al., 2000a
	0.16 mg/kg	+	Dekeyne et al., 2000a
	0.63 mg/kg	0	Dekeyne et al., 2000a
Ipsapirone	200 ng CNS	+	Higgins et al., 1988, 1992
	0.5 mg/kg	0	Costall et al., 1992
	1 or 5 mg/kg	+	Costall et al., 1992
	5 mg/kg	+	Picazo et al., 1995
	2.5 and 10 mg/kg	0	Picazo et al., 1995
	0.2 μg/μl dorsal raphe nucleus	+	Picazo et al., 1995
	0.2 μg/μl intrahippocampal	0	Picazo et al., 1995
	5 mg/kg	0	To and Bagdy, 1999
Gepirone	5.0–10.0 mg/kg	+	Dunn et al., 1989
	0.2–1 μg CNS	+	Higgins et al., 1992
Lesopitron (E-4424)	0.001, 0.01 or 0.05 mg/kg	+	Costall et al., 1992
S20499	0.04, 0.2 or 1 mg/kg	+[a]	File and Andrews, 1994
MKC-242	0.1–0.5 mg/kg	+	Abe et al., 1996
S15535	0.63, 2.5 or 10.0 mg/kg	+	Dekeyne et al., 2000a

+: Anxiolytic. –: Anxiogenic. 0: No effect.
[a] Diazepam-withdrawn rat.

both time spent in social interaction and concurrent locomotion under HU and LF conditions, a profile more consistent with sedation than anxiogenesis (Kennett et al., 2000). However, SB-243213, a selective 5-HT_{2C} receptor inverse agonist exhibited anxiolytic-like activity in the social interaction test (Wood et al., 2001).

The function of the 5-HT_{2B} receptor is unclear due to a paucity of selective agents. BW 723C86, a 5-HT_{2B} receptor agonist, administered subcutaneously or microinjected bilaterally into the medial amygdaloid nuclei, increased the total interaction time in rat social interaction test, which was prevented by pretreatment with the 5-$HT_{2C/2B}$ receptor antagonist, SB 200646A (Kennett et al., 1996a; Duxon et al., 1997).

7.3. Antidepressant and antihistaminergic drugs

Antidepressant drugs have been found to have clear anxiolytic effects clinically, but only after chronic treatment. After acute administration, they have anxiogenic effects both clinically and in the social interaction test (see Section 5.5). However, even after chronic treatment, it has been surprisingly difficult to demonstrate anxiolytic actions of antidepressant drugs in any animal tests of anxiety (File et al., 1999; To and Bagdy, 1999; To et al., 1999). The first report of an anxiolytic effect is following chronic administration of paroxetine (Lightowler et al., 1994). Later, it has been observed that concurrent 5-HT_{1A} receptor blockade by WAY 100635 reduced the time to onset of anxiolysis seen with paroxetine alone (from 21 to 7 days), suggesting that the anxiolysis induced by the combination is not attributable to postsynaptic 5-HT_{1A} receptor activation rather attributable to a CNS adaptive response (Duxon et al., 2000). Neonatal clomipramine treatment did not alter social interaction when rats were tested as adults (File and Tucker, 1983a). However, prenatal clomipramine treatment resulted in an anxiolytic profile when male and female rats were tested as adolescents (File and Tucker, 1983a, 1984a).

The tricyclic antihistaminic drug, Ketotifen, and its analogue, HF200-184, increased social interaction with no change in locomotor activity. However, other analogues like HE36-953, SDZ206-703 and SDZ209-321 failed to modify social interaction in the rat (Barnes et al., 1990a,b; Costall et al., 1990c).

7.4. Herbal extracts

Several plants have been screened for their possible anxiolytic activities by Bhattacharya's group. It has been observed that bioactive glycowithanolides isolated from *Withania somnifera* roots, a herbal psychotropic formulation, BR-16A (Mentat) and 50% ethanolic extract of Indian *Hypericum perforatum* have all exhibited significant anxiolytic effect in the rat social interaction test (Bhattacharya, 1994; Bhattacharya et al., 2000; Kumar et al., 2000).

8. Brain regions mediating effects in the social interaction test

8.1. Amygdala

The amygdala has long been considered to be an important structure mediating changes in anxiety and following injection of the specific neurotoxin 5,7-dihydroxytryptamine, resulting in 80% depletion of 5-HT, rats spent significantly less time in social interaction and had reduced locomotor activity (File et al., 1981). The lesion, therefore, seemed to be having a nonspecific effect of decreasing spontaneous behaviours. After direct injection into the amygdala, Higgins et al. (1991) observed that 5-HT_3 receptor antagonists significantly increased social interaction in rats, indicating possible anxiolytic effects of these compounds in this brain region. Injection of flurazepam into an ill-defined region of the amygdala complex also had significant anxiolytic effects (Higgins et al., 1991). The amygdala is a multinuclear complex, and there are important functional differences between the different nuclei, which receive different inputs, and have different outputs. With increasing knowledge of these nuclei, it has become important to study the roles of the separate nuclei, and this has been achieved using central drug infusions into localised areas.

8.1.1. Basolateral nucleus

Infusion of the benzodiazepine, midazolam, into the basolateral nucleus had anxiolytic effects in the social interaction test (Gonzalez et al., 1996), and flumazenil or bicuculline infused into this region were able to antagonise the anxiolytic effect of systemic chlordiazepoxide (Sanders and Shekhar, 1995a). There would seem to be high intrinsic GABAergic tone in this area because infusion of the $GABA_A$ receptor agonist, muscimol, was without effect, whereas the $GABA_A$ receptor antagonists, bicuculline and picrotoxin, had anxiogenic effects (Sanders and Shekhar, 1995b). Repeated infusions of bicuculline into this area led to sensitisation of the anxiogenic effect (Sanders and Shekhar, 1995b) and to an anxiogenic response to sodium lactate that was not seen in nonsensitised rats (Sajdyk and Shekhar, 2000). The $GABA_B$ and glycine receptors in this region appeared to play no role in modulating anxiety since microinjections of the $GABA_B$ receptor agonist, baclofen, the $GABA_B$ receptor antagonist, 2OH-saclofen, and the glycine receptor antagonist, strychnine, were without effect (Sanders and Shekhar, 1995b).

The benzodiazepines may be exerting their anxiolytic effects in this region by inhibiting the 5-HT system. Stimulation of the postsynaptic 5-HT_{1A} receptors in the basolateral nucleus by microinjection of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, had a significant anxiogenic effect (Gonzalez et al., 1996). There may also be an interaction between the GABA and excitatory amino acid systems because NMDA receptor antagonists injected into the basolateral nucleus blocked the anxiogenic effects of bicuculline (Sajdyk and Shekhar, 1997a). However, these antagonists themselves have anxiolytic effects (Sajdyk and Shekhar, 1997b) and, thus, it is possible that the antagonism is a functional one and does not necessarily reflect direct pharmacological antagonism. The basolateral nucleus is also a site of the anxiolytic effects of neuropeptide Y (Sajdyk et al., 1999b). The effects are mediated via the neuropeptide Y Y_1 receptor since injections of the neuropeptide Y Y_1 receptor antagonist into this region blocked the anxiolytic-like effects of neuropeptide Y in the social interaction test (Sajdyk et al., 1999b). In contrast, activation of the neuropeptide Y Y_2 receptor by directly infusing the selective neuropeptide Y Y_2 receptor agonist significantly decreased social interaction time in rats which could be blocked by pretreatment of the anxiolytic alprazolam (Sajdyk et al., 2002).

8.1.2. Central nucleus

Injections of morphine into the central nucleus of the amygdala had anxiolytic effects (File and Rodgers, 1979), as did injections of muscimol (Sanders and Shekhar, 1995b). In contrast to the basolateral nucleus, the endogenous GABAergic tone in the central nucleus seems to be low because $GABA_A$ receptor antagonists were without effect (Sanders and Shekhar, 1995b). Neuropeptide Y was without effect in the central nucleus (Sajdyk et al., 1999b), as was unilateral injection of the 5-HT_{2C} receptor agonist mCPP (Whitton and Curzon, 1990).

8.1.3. Medial nucleus

Injection of a 5-HT_{2B} receptor agonist into the medial amygdala had an anxiolytic effect that was antagonise by a 5-$HT_{2B/2C}$ receptor antagonist (Duxon et al., 1997). In contrast to the effect seen in the central nucleus, morphine injected into the medial amygdala had an anxiogenic effect (File and Rodgers, 1979).

8.2. Hippocampus

The hippocampus is also a region that has long been implicated in the control of anxiety responses. Marked anxiolytic effects were seen in the social interaction test of rats with cytotoxic lesions of the hippocampus (Deacon et al., 2002). It is important to distinguish between the dorsal and ventral hippocampus, since these areas receive different inputs and may play distinct roles in anxiety. The dorsal raphe nucleus mainly projects to the ventral hippocampus, as well as to the basolateral nucleus of the amygdala, the cortex and basal ganglia (Azmitia and Segal., 1978). The dorsal raphe nucleus plays an important role in mediating anxiety (see later section), but its projection site to the ventral hippocampus may not be of prime importance. Hogg et al. (1994) found no effects in the social interaction test of the 5-HT_{1A} receptor agonist, 8-OH-DPAT when injected bilaterally into the ventral hippocampus. However, exposure

of rats to social interaction resulted in an increase in extracellular 5-HT and cAMP in the ventral hippocampus as measured by in vivo microdialysis (Cadogan et al., 1994), which suggests that other 5-HT receptor subtypes may be important in this region. In addition, decreases in social interaction with enhanced levels of aggression have been observed in rats with neonatal ibotenic acid lesion of the ventral hippocampus (Sams-Dodd et al., 1997; Becker et al., 1999), which indicate that these changes are due to lesion-induced impairments in neurodevelopmental processes at an early stage of ontogenesis (Becker et al., 1999).

8.2.1. Dorsal hippocampus

Bilateral injections of the benzodiazepine, midazolam, had an anxiolytic effect that was reversed by a silent dose of the benzodiazepine receptor antagonist, flumazenil (Gonzalez et al., 1998b). Rats withdrawn from chronic diazepam treatment were significantly more anxious in the social interaction test and had significantly greater K^+-evoked release of [^{3}H]5-HT from slices of dorsal and of ventral regions of the hippocampus (Andrews et al., 1997). Injection of the 5-HT_{2C} receptor agonist, mCPP, into the dorsal hippocampus had an anxiogenic effect (Whitton and Curzon, 1990). Injection of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, also had an anxiogenic effect, as did injection of tertatolol (Andrews et al., 1994). Tertatolol has antagonist actions at both β-adrenoceptors and 5-HT_{1A} receptors, so this effect of tertatolol is somewhat surprising and suggests that tertatolol may have some partial agonist properties. The anxiogenic effect of 8-OH-DPAT was confirmed by File et al. (1996), and this was reversed with a silent dose of the specific 5-HT_{1A} receptor antagonist, WAY 100,635, thus, confirming the mediation by 5-HT_{1A} receptors. Nicotine also has an anxiogenic effect when injected into the dorsal hippocampus and, interestingly, this was also antagonised by WAY 100,635, but not by the muscarinic M_1 receptor antagonist, pirenzepine (Kenny et al., 2000). These effects were observed in the high light, familiar test condition which generates a moderate level of anxiety. File et al. (1998) showed that the anxiogenic effects of nicotine, both after systemic injection and after injection into the dorsal hippocampus, could only be observed under conditions of moderate anxiety. In the low light, familiar test condition, which generates the lowest level of anxiety and in the high light, unfamiliar test condition, which generates the highest level of anxiety, nicotine was without effect. This suggests that the endogenous cholinergic and/or serotonergic tone in the dorsal hippocampus can determine nicotine's effects (File et al., 2000). Under conditions of low anxiety (low light, familiar test condition), the muscarinic M_1 receptor antagonist, pirenzepine, and the nicotinic receptor antagonist, mecamylamine, both had anxiogenic effects (File et al., 1998). This suggests that there is an endogenous cholinergic tone mediating an anxiolytic action through postsynaptic muscarinic M_1 receptors; the action of mecamylamine is most likely mediated on its action at presynaptic receptors to reduce acetylcholine release. The muscarinic M_2 receptor antagonist, gallamine, was without effect, suggesting that the muscarinic M_2 receptors in this brain region do not play a role in mediating the effects of endogenous tone.

8.3. Lateral septum

Bilateral injections of the specific 5-HT neurotoxin, 5,7-dihydroxytryptamine, into the lateral septum resulted in a significant anxiolytic profile, similar to that seen with benzodiazepines (Clarke and File, 1982). The importance of the 5-HT system for this anxiolytic effect was confirmed by the lack of effect of the noradrenergic neurotoxin, 6-hydroxydopamine (Clarke and File, 1982). Recent studies have shown that the 5-HT_{1A} receptors in this region play an important role, and Cheeta et al. (2000a) found that the 5-HT_{1A} receptor agonist had an anxiogenic effect when injected into the lateral septum. Cheeta et al. (2000b) also found that 5-HT_{1A} receptors also mediate the anxiogenic effects of nicotine injected into this area. Overall, the results of the lesion and drug administration studies suggest that there is a tonic 5-HT tone at 5-HT_{1A} receptors in the lateral septum that exerts an anxiogenic effect. The anxiogenic effect of nicotine in the lateral septum could be reversed by a silent dose of the noncompetitive nicotinic receptor antagonist, mecamylamine (Ouagazzal et al., 1999). However, at lower doses, mecamylamine had an intrinsic anxiolytic effect and at higher doses an intrinsic anxiogenic effect. This suggests the existence of at least two different populations of nicotinic receptors in this region at which there is endogenous cholinergic tone exerting opposite behavioural effects.

8.4. Hypothalamus

The hypothalamus has been implicated in the regulation of autonomic, neuroendocrine and behavioural expressions of emotions. Previous reports suggest that the GABA neurones in the dorsomedial hypothalamus regulate a constellation of physiologic and behavioural responses associated with anxiety and stress. Blocking and enhancing $GABA_A$ receptor function in the dorsomedial hypothalamus of rats respectively elicit anxiogenic and anxiolytic effects in the social interaction test (Shekhar and Katner, 1995). It was proposed that change in 5-HT turnover in the midbrain and hypothalamus is possibly linked to the anxiogenic effects of ACTH (File and Vellucci, 1978) and anxiolytic effects of chlordiazepoxide (Vellucci and File, 1979). Later studies indicated that hypothalamic catecholamines might not be involved in the aversive responses elicited in this region. 6-hydroxydopamine-induced lesions of the paraventricular nucleus of the hypothalamus did not alter total scores in the social interaction, except increased vigorous contact, similar to behaviour previously observed in younger rats (Kellogg et al., 1993). In addition, rats sub-

jected to the social interaction test showed no change in tissue catecholamine levels in the dorsomedial hypothalamus at 24 h posttest (Sajdyk et al., 1997).

8.5. Frontal cortex

It has been proposed that the reciprocal neural circuits linking the medial prefrontal cortex, the extended amygdala and the hypothalamus play a crucial role in mediating fear and responses to stress. Early studies had reported increased social behaviour following lesions of the medial prefrontal cortex (de Bruin et al., 1983; Holson and Walker, 1986). A more recent study investigated the effect of transection of the medial prefrontal cortex in the social interaction test, where it was found to have an anxiolytic action (increasing social interaction, without changing motor activity) and was able to antagonise the anxiogenic effect of amphetamine (Gonzalez et al., 2000).

8.6. Raphe nuclei

As has been reviewed in earlier sections, both systemically and centrally administered 5-HT receptor ligands have been shown to modulate anxiety as measured in the social interaction test. The 5-HT cell bodies are located in the midbrain raphe nuclei, from where they send projections to several terminal areas. The dorsal raphe nucleus primarily sends projections to the ventral hippocampus, the basolateral nucleus of the amygdala and the frontal cortex. The median raphe nucleus provides the main 5-HT input into the dorsal hippocampus. Large lesions of both nuclei with 5,7-dihydroxytryptamine reduced social interaction in all four test conditions, but also reduced locomotor activity, suggesting that this lesion caused marked hypoactivity (File and Deakin, 1980).

8.6.1. Dorsal raphe nucleus

The importance of the dorsal raphe nucleus in mediating changes in anxiety, as measured in the social interaction test, was first demonstrated by File et al. (1979b). They showed that microinjection of the 5-HT neurotoxin into the dorsal raphe nucleus had a specific anxiolytic effect, similar to that seen with the benzodiazepines. Furthermore, the lesioned rats no longer responded to the anxiogenic effects of ACTH. This suggested the importance of the 5-HT neurones in this area. However, the dorsal raphe nucleus also contains some noradrenergic input from the locus coeruleus and injection of the neurotoxin 6-hydroxydopamine dramatically increased social interaction in all four of the test conditions. This pattern of results resembled that seen with the barbiturates, rather than the benzodiazepines.

Hindley et al. (1985) found that injections of the β-carboline, β-CCM, which is a benzodiazepine receptor inverse agonist, into this region decreased social interaction, indicating an anxiogenic effect. Furthermore, this anxiogenic effect was reversed by co-administration of the benzodiazepine receptor antagonist, flumazenil. The importance of the 5-HT_{1A} autoreceptors in the dorsal raphe nucleus was shown by Higgins et al. (1992), who showed that injection of the 5-HT_{1A} receptor agonists, 8-OH-DPAT, buspirone, ipsapirone and gepirone, had anxiolytic effects. Low doses of the 5-$HT_{1B/2C}$ receptor agonist, mCPP, were without effect in this region and high does produced hypoactivity. At a comparatively high dose the 5-HT_{1B} receptor agonist, CGS 12066B, had an anxiolytic effect, but this may have been die to an action at 5-HT_{1A} receptors, because the drug loses specificity at high doses. Confirmation of the anxiolytic effect of 8-OH-DPAT when injected into the dorsal raphe nucleus came from Hogg et al. (1994), who showed that the anxiolytic effect could be blocked by co-administration of the 5-HT_{1A} receptor antagonist, tertatolol. Cheeta et al. (2001b) showed that nicotine injected into the dorsal raphe nucleus had an anxiolytic action that seemed to be mediated by 5-HT_{1A} receptors, since it could be antagonised by the 5-HT_{1A} receptor antagonist, WAY 100,635. Dihydro-β-erythroidine antagonised the anxiogenic effect of nicotine in this region, suggesting that the nicotinic subreceptors mediating this anxiolytic effect are the $á_4β_2$ subreceptors (Cheeta et al., 2001d). Not only does the dorsal raphe nucleus mediate nicotine's anxiolytic effects; it is also the area that mediates the development of tolerance to this effect following chronic nicotine treatment (Cheeta et al., 2001b). When rats were withdrawn from 7 days of nicotine injections, there was an anxiogenic response that could be reversed by nicotine administered systemically, or into the dorsal raphe nucleus (Cheeta et al., 2001b).

8.6.2. Median raphe nucleus

The median raphe nucleus seems equally important to the anxiolytic action of 5-HT_{1A} receptor agonists, and injection of 8-OH-DPAT into this region had anxiolytic effects (Andrews et al., 1994) that could be antagonised by WAY 100,635 (File et al., 1996). In light of these results, it is somewhat surprising that 5,7-dihydroxytryptamine lesions of the median raphe nucleus were without significant effect (File et al., 1979b). Certainly, this nucleus is also important to the anxiolytic effects of benzodiazepines. Injections of midazolam had anxiolytic effects that were antagonised by a silent dose of fluamzenil (Gonzalez et al., 1998b).

8.6.3. Locus coeruleus

The locus coeruleus contains noradrenergic cell bodies that give rise to projections throughout the cerebral and cerebellar cortices. In contrast to the results observed in the dorsal raphe nucleus, 6-hydroxydopamine lesions of the locus coeruleus were without effect in the social interaction test (Crow et al., 1978; File et al., 1979a), and animals with this lesion showed a normal response to ACTH (File et al., 1979a). These results, together with the effects of systemically administered drugs, suggests that the noradrenergic system plays a much less important role in control-

ling behaviour in the social interaction test than does the 5-HT system.

9. Conclusions

The social interaction test of anxiety has proved extremely useful in detecting both anxiolytic and anxiogenic effects of environmental factors and systemically administered drugs and peptides. It has also proved invaluable in unravelling the neural basis of anxiety. Most of the research over the past 25 years has been on the state of anxiety that is induced by the test itself. This is thought to mimic the state of anxiety most similar to that experienced in generalised anxiety disorder. However, an important advance for the future will be to use the test to address the question of the genetic basis of anxiety. This has already started with the characterisation of various knock-out or genetically modified mice. However, other approaches are possible and it is possible to study gene modification after exposure to the various conditions of the social interaction test, with and without drug treatment. We hope the next quarter of a century of research using this test will prove as interesting and fruitful as the last.

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