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Ketamine administration disturbs behavioural and distributed neural correlates of fear conditioning in the rat

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Abstract

The neurotransmitter glutamate and its associated receptors perform an important role in the brain circuitry underlying normal fear processing. The glutamate NMDA receptor, in particular, is necessary for the acquisition and recollection of conditioned-fear responses. Here the authors examine how acute blockage of the NMDA receptor with sub-anaesthetic doses of ketamine affects behavioural assays of fear-conditioned stress (e.g. freezing) and cFos expression in a network of brain areas that have previously been implicated in fear processing. Fear-conditioned rats displayed significantly more freezing behaviour than non-conditioned controls. In fear-conditioned rats that also received ketamine, this conditioning effect was largely neutralised. Fear conditioning also led to increased cFos expression in various areas central to fear processing, including the basolateral nucleus of the amygdala, the paraventricular nucleus of the hypothalamus and the anterior cingulate. Ketamine abolished such increases in cFos expression in most brain areas investigated. The present study therefore demonstrates that systemic ketamine administration in rats interferes with fear conditioning on a behavioural level and in a network of brain regions associated with fear and anxiety. The combination of ketamine and fear conditioning may therefore provide a useful model of abnormal fear processing, as observed in certain psychiatric conditions.
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Keywords: Amygdala; Anterior cingulate; cFos; Fear conditioning; NMDA receptor

1. Introduction

Classical fear conditioning is a technique generally used to explore fear circuits in the brain. It involves learning an association between a neutral conditioned stimulus and an aversive unconditioned stimulus (Walker and Davis, 2002). The neural pathways underlying fear conditioning, and more generally, fear processing, have been thoroughly investigated in the rat, with the primary focus being the amygdala (Davis et al., 1994; Maren and Fanselow, 1996; LeDoux, 1998, 2000; Maren, 2001).

Previous studies have shown that NMDA receptors in the amygdala are essential for long-term potentiation (LTP), a process that underlies fear learning (Li et al., 1995; Lee et al., 2001). Amygdalar NMDA receptors are necessary for the convergence and association of the unconditioned and conditioned stimuli (Walker and Davis, 2002). Kim and McGaugh (1992) for example, examined how injecting various NMDA antagonists (AP5, MK-801, CPP) into the rat amygdala altered conditioned-fear behaviour in an inhibitory avoidance task. While acquisition of conditioned responses remained intact, deficits in inhibitory avoidance were noted 48 h later. It has been hypothesized that temporally precise fear responses to specific threats are mediated by the amygdala, whereas sustained anxiety responses that persist beyond the immediate threat are mediated, at least in part, by structures such as the anterior cingulate, nucleus accumbens, orbitofrontal cortex and insula (Davidson and Irwin, 1999; Cardinal et al., 2002; Walker and

Davis, 2002). In one study, for example, the pairing of anterior-cingulate stimulation with an auditory tone produced conditioned-fear responses in the rat (Tang et al., 2005). These responses were then blocked by the infusion of an NMDA-receptor antagonist into the amygdala. Such studies indicate a functional relationship between the anterior cingulate and the amygdala during fear processing.

To further investigate the functional basis of normal and altered fear processing, we induced a state of fear-conditioned stress (Suzuki et al., 2002) – a state dependent on the environmental context in which classical conditioning occurs – in the rat. We then examined behavioural (e.g. freezing) and neural (cFos expression) assays of fear conditioning following the (systemic) administration of ketamine, an NMDA antagonist, and in control animals. Based on available knowledge of the processing regions likely to be involved in fear and stress responses (LeDoux, 1998; Davidson and Irwin, 1999; Cardinal et al., 2002; Walker and Davis, 2002), we identified several candidate brain regions for investigation, including the amygdala and anterior cingulate. We hypothesized that ketamine would abolish neural and behavioural responses associated with fear conditioning, thereby inducing a state of abnormal fear processing which may prove useful in developing animal models of certain psychiatric conditions, such as schizophrenia.

2. Materials and methods

2.1. Animals

All animals were cared for in accordance with the principles laid down by the European Communities Council Directive (1986) for the Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (86/EEC). Sprague–Dawley rats ($n=24$) weighing between 225–250 g were obtained from the central animal facility (Groningen, The Netherlands) and were housed individually in a temperature (± 23 °C) and humidity controlled (40% to 60%) environment. Food and water were delivered ad libitum. After arrival from the animal breeding facility, rats were allowed to acclimatize for two to three days. They were then handled daily for five days in order to eliminate handling stress as a confounding variable.

The rats were divided into four groups: fear conditioned and ketamine, fear conditioned and saline (fear conditioning), no fear conditioning and ketamine (ketamine), and no fear conditioning and saline (control).

2.2. Drugs and injection paradigm

Ketamine hydrochloride was obtained from Sigma (Germany). Ketamine injections (16 mg/kg, s.c.) and saline shams were administered half an hour before fear conditioning for the first 2 days (i.e. only during the actual conditioning phase of the experiment). Previous observations in our lab (Imre et al., 2006), as well as other studies (Pallares et al., 1995), show that half an hour is sufficient for the ketamine-induced alterations in locomotor activity to normalise. All injections were omitted on the third day of conditioning testing to avoid unnecessary drug interaction with behavioural measurements. The ketamine dose was determined empirically (i.e. it was the highest dose possible that did not affect locomotor behaviour).

2.3. Shock paradigm

The paradigm according to Suzuki et al. (2002) was followed with some minor modifications. We chose this more-stressful protocol compared to traditional fear conditioning studies because we wanted to induce persistent changes at both the biochemical and behavioural level.

The rats were taken out of their home cage and placed individually in the shock box. This was a specially constructed wooden container with a floor made of a metal grid. A central computer controlled the current and noise emission, making use of a program that was specially developed for this study (N594 version 2.00, Rijksuniversiteit Groningen, The Netherlands, 2002). Rats destined to undergo fear conditioning were then subjected to a shock (1.5 mA) that was paired with a noise (60 dB tone) for two days (Fig. 1). This shock intensity was based on a pilot study (Pietersen et al., 2006) indicating that 1.0 and 1.5 mA shocks induced comparable stress levels (corticosterone and behaviour), but also showing that the latter shock intensity was clearly superior in terms of variability of all incurred stress parameters.

One shock session consisted of a 1-min period. During the first 30 s the noise was emitted. Within the second half (15 s) of this 30-s period, the shock was delivered. The last 30 s served as a rest period. All sessions took place in the morning and were repeated consecutively ten times per day, resulting in one session lasting 10 min in total. Control rats followed the same routine with noise emission, but without experiencing any shocks.

On the third day, the same procedure was followed, but without administering shocks. The behaviour following fear-conditioned stress was then noted for 30 min after the last noise session. This time point was chosen as it has been shown that freezing behaviour persisted long after the last noise session (Suzuki et al., 2002).

2.4. Behavioural observation

Behaviours were recorded for each rat by means of a video camera (Philips Explorer Camcorder) directly after the last noise session. They were then subsequently analysed with the aid of the computer program, The Observer (Noldus version 3.0, The Netherlands). An independent observer unaware of experimental conditions noted freezing, grooming, rearing and resting. Freezing was denoted as an absence of any movement except that needed for respiration and whisker twitching. Rearing was defined as the raising of the body onto the hind legs, while resting served as a default state whereby none of the other behaviours were being displayed. The freezing behaviour, as well as being a behavioural expression of stress, is also the main determinant of successful fear conditioning. Rearing and grooming were denoted as anxiolytic behaviours (Morrow et al., 2002; Sharp et al., 2002; Daniels et al., 2004).

2.5. Perfusion and preparation for cFos staining

Half an hour after the end of the behavioural observation, the rats were perfused trans-cardially with 4% paraformaldehyde (Merck, Germany) for 20 min. The brains were then removed and placed into 4% paraformaldehyde, and kept at 6 °C for 2 days. Thereafter, they were transferred into 0.02 M potassium phosphate buffered saline (PBS; pH 7.4) with 1% sodium azide (Boom, Meppel, The Netherlands) to prevent bacterial growth and were stored at 6 °C. In preparation for cFos staining, whole brains were dehydrated in a 30% sucrose solution overnight and subsequently frozen with gaseous CO_2 at −80 °C. The brains
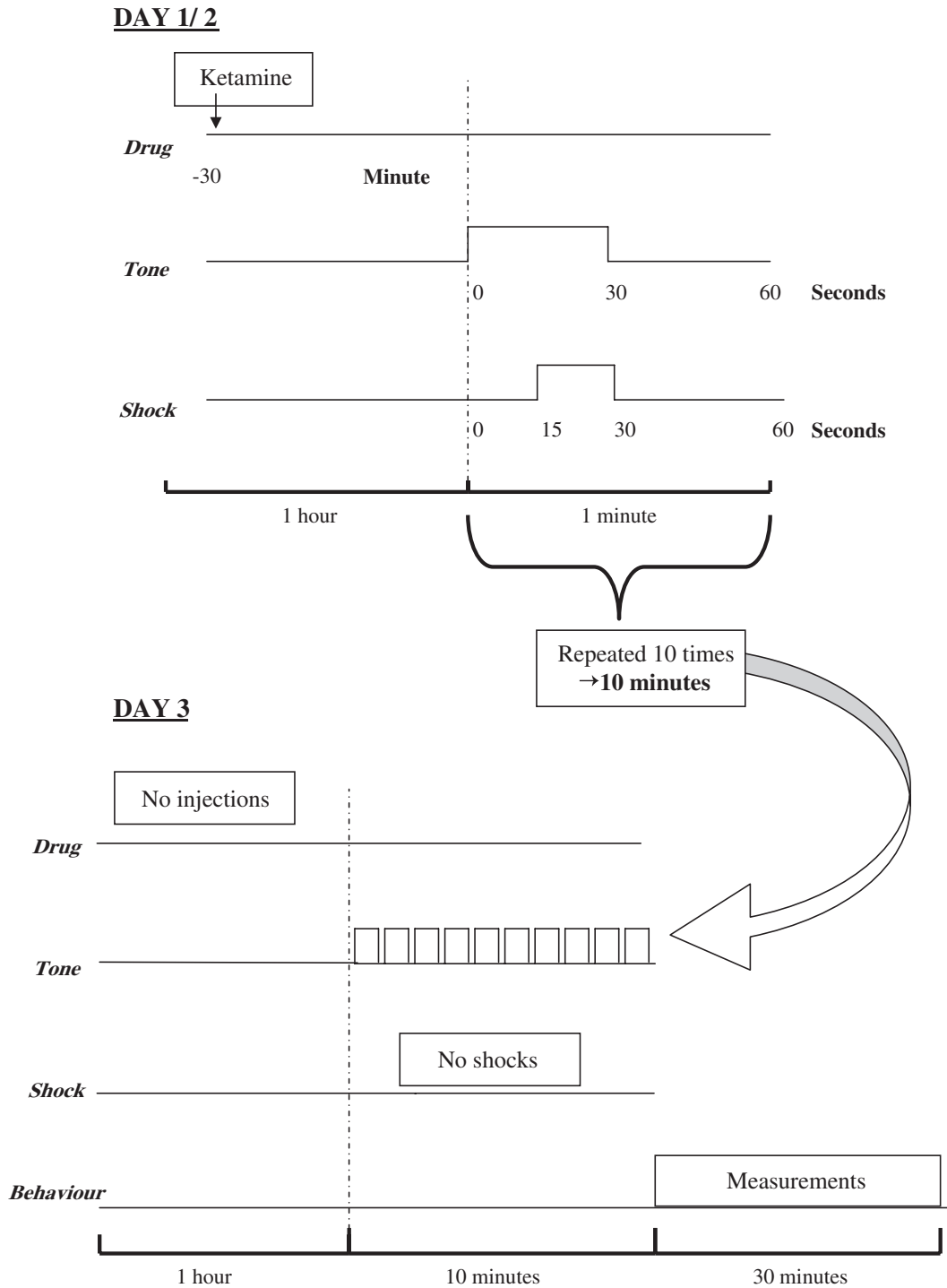
Fig. 1. Fear conditioning schedule. Injection and shock paradigm. This diagram represents the methodology employed during fear conditioning. On day one and two, the same protocol is followed with only a sound given during the first 15 s. In the next 15 s, the sound is presented in combination with a shock (1.5 mA). The last 30 s serves as a rest period. On day 3, no shocks or injections are given, although the animal follows the same protocol. This 1-min session is repeated 10 times per day in succession. The behaviour is noted for 30 min after the last test session on the third day.

were cut using the Leica CM 3050 cryostat machine at 40 μm thin slices and stored at 6 °C in 0.02 M PBS buffer (pH 7.4).

2.6. cFos staining: immunocytochemistry

Coronal cryostat sections of 40 μm were collected in 0.01 M Tris buffered saline (TBS, pH 7.4) and rinsed 3×5 min. After pre-incubation with 0.3% H_2O_2 (10 min, in 0.01 M TBS, pH 7.4), the sections were washed with 0.01 M TBS (4×5 min, pH 7.4) and incubated with a rabbit polyclonal antibody raised against cFos (Ab-5 Oncogene Research Products, Calbiochem, 1:10.000 in 0.01 M TBS-Triton 0.01%, 4% normal goat serum) for 24–30 h at room temperature. Subsequently, the sections were washed in 0.01 M TBS (8×5 min, pH 7.4) and incubated for 2 h at room temperature with biotinylated goat anti-Rabbit IgG (Vector, 1:1000 in 0.01 M TBS). After rinsing with 0.01 M TBS (6×5 min, pH 7.4), the immunoreactivity was visualized with a standard ABC method (Vectastain ABC kit, Vector, (1 drop A+1 drop B)/20 ml TBS for 2 h). After washing with TBS 0.01 M (6×5 min, pH 7.4) the peroxidase reaction was developed with a di-aminobenzidine (DAB)-nickel solution and 0.3% H_2O_2 (0.5 mg DAB/ml Distilled water; 1.0% nickel ammonium sulphate (NAS) in 0.1 M sodium acetate (NaAc, pH 6.0). To stop the reaction, the sections were washed with 0.1 M NaAc, pH 6.0 (3×5 min) and then 0.01 M TBS (3×5 min, pH 7.4) and were subsequently mounted on gelatin-coated slides, air dried, dehydrated, and coverslipped with DePeX (Gurr) (Boom, Meppel, The Netherlands).

The area of the region of interest was measured and, after background correction, the number of immunopositive nuclei was quantified using a computerized image analysis system (Leica Qwin version 2.3, Leica Microsystems Imaging Solutions). The average number of cFos immunoreactive cells was calculated and expressed as number of positive nuclei or counts/area $(0.1mm^2)$.

Areas included in the cFos analysis were: the paraventricular nucleus, central and basolateral amygdala nuclei, nucleus accumbens, dorsal raphe, locus coeruleus, anterior cingulate and dentate gyrus. The Swanson (1992) co-ordinates are given in Table 1 as millimetres from Bregma.

2.7. Statistics

A two-way ANOVA was utilized in the program JMP 4.0.4 (SAS institute). When the ANOVA indicated a "fit" with a

Table 1
Brain areas: Swanson (1992) rostral–caudal co-ordinates

Area	mm from Bregma
Paraventricular nucleus	−1.53 to −2.00
Central nucleus of amygdala	−2.45 to −2.85
Basolateral nucleus of amygdala	−2.45 to −2.85
Nucleus accumbens	+2.80 to +2.15
Dorsal raphe	−7.10 to −8.60
Locus coeruleus	−9.60 to −10.10
Anterior cingulate	+2.80 to +2.15
Dentate gyrus	−2.45 to −2.85

p value < 0.05, a determination for the dependent variables fear conditioning vs. no fear conditioning and saline vs. ketamine treatments was performed. In addition, an interaction factor was included. When appropriate (i.e. a significant interaction factor of $p < 0.05$), individual group comparisons were made using a Tukey's HSD. All behavioural data analysis, as well as the paraventricular nucleus and dorsal raphe data, were performed on log (base 10) transformed data via JMP 5.1 statistical package in order to normalise the distribution.

3. Results

3.1. Behaviour

The results of the ANOVA on frequency, percentage total duration of the half-hour session and the mean time that the animal displayed certain behaviour are analysed and are stated here. Results of the post hoc test for individual comparisons are represented in Fig. 2.

3.1.1. Frequencies (Fig. 2a)
Fear-conditioned stress increased freeze frequency in the presence of saline, but had no effect in combination with ketamine ($F_{1, 20} = 11.55$; $p < 0.01$). Ketamine also decreased rest frequency in the presence of fear conditioning ($F_{1, 20} = 11.54$; $p < 0.01$).

3.1.2. Percentage total duration (Fig. 2b)
In terms of percentage total duration, fear-conditioned stress again increased freeze duration, whereas in combination with ketamine this effect was reduced to control levels ($F_{1, 20} = 24.53$; $p < 0.0001$). Ketamine itself decreased freezing duration with fear conditioning, but increased freeze duration without fear conditioning. A significant interaction effect was noted in resting duration ($F_{1, 20} = 6.56$; $p < 0.05$), but no individual differences were noted between groups. Fear-conditioned stress also decreased total grooming duration ($F_{1, 20} = 10.07$; $p < 0.01$), while ketamine in combination with fear conditioning reversed this effect ($F_{1, 20} = 4.88$; $p < 0.05$). No interaction effect was noted here.

3.1.3. Means (Fig. 2c)
Ketamine had a main effect on the mean resting period ($F_{1, 20} = 15.55$; $p < 0.001$), but no effects were noted in terms of freezing behaviour. Grooming had significant interaction effects ($F_{1, 20} = 15.16$; $p < 0.001$), with fear-conditioned stress decreasing mean grooming time in the presence of saline, but not in the presence of ketamine. Ketamine increased grooming under fear conditioning, but had no effect without fear conditioning.

3.2. cFos expression

Results of Tukey's post hoc test for individual comparisons are represented in Fig. 3. The two-way ANOVA revealed a fear conditioning main effect in the dorsal raphe ($F_{1, 16} = 10.30$; $p < 0.01$), central amygdala ($F_{1, 19} = 5.77$; $p < 0.05$) and basolateral

amygdala ($F_{1, 19}=23.43$; $p<0.0001$), while ketamine had a significant main effect on cFos in the basolateral amygdala ($F_{1, 19}=19.15$; $p<0.001$). A significant interaction between fear conditioning and ketamine treatment was found in the anterior cingulate ($F_{1, 19}=8.41$; $p<0.01$), locus coeruleus ($F_{1, 17}=4.36$;

$p=0.05$), nucleus accumbens ($F_{1, 19}=9.53$; $p<0.01$) and paraventricular nucleus ($F_{1, 19}=23.46$; $p<0.001$). In the dentate gyrus, ketamine decreased cFos expression ($F_{1, 20}=17.44$; $p<0.001$). Typical areas analysed and cFos staining are shown in Fig. 4.
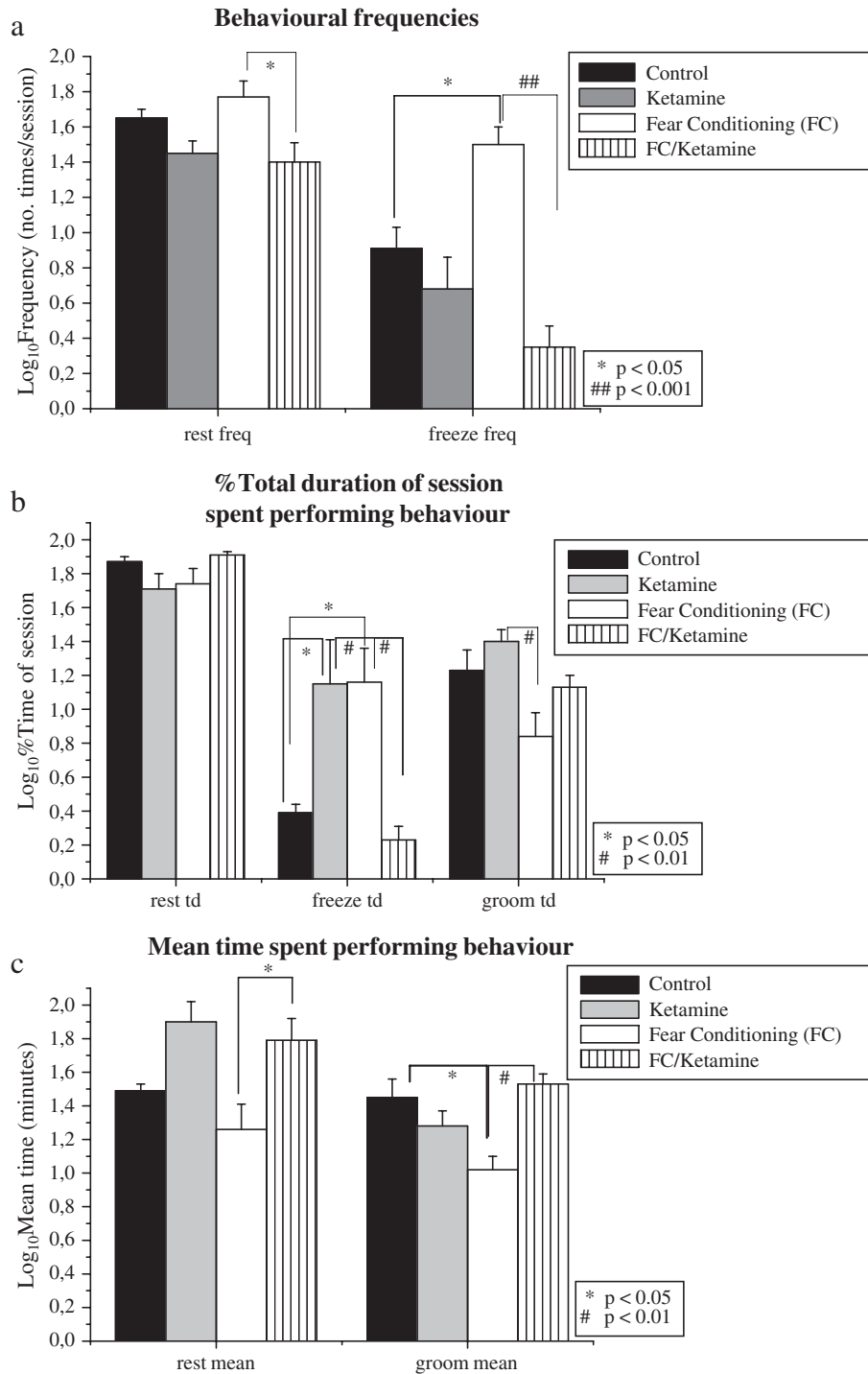

Fig. 2. Behavioural data. Significant differences shown on the graphs are according to Tukeys' post hoc test. Significant differences between non-related groups (i.e. no fear conditioning and saline vs. fear conditioned and ketamine) are not shown. Fear conditioning (a) increased freeze frequency ($p=0.028$), (b) increased total duration of freezing ($p=0.023$), and (c) decreased the mean time spent grooming ($p=0.012$). Ketamine reversed (a) the effect of fear conditioning on rest ($p=0.027$) and freeze frequency ($p<0.001$), (b) total duration of freezing ($p=0.005$), and (c) mean time spent resting ($p=0.025$) and grooming ($p=0.003$). Ketamine also (b) increased the total duration of freezing in the presence of saline ($p=0.024$), but not in the presence of fear conditioning ($p=0.005$). (freq = frequency, td = total duration).
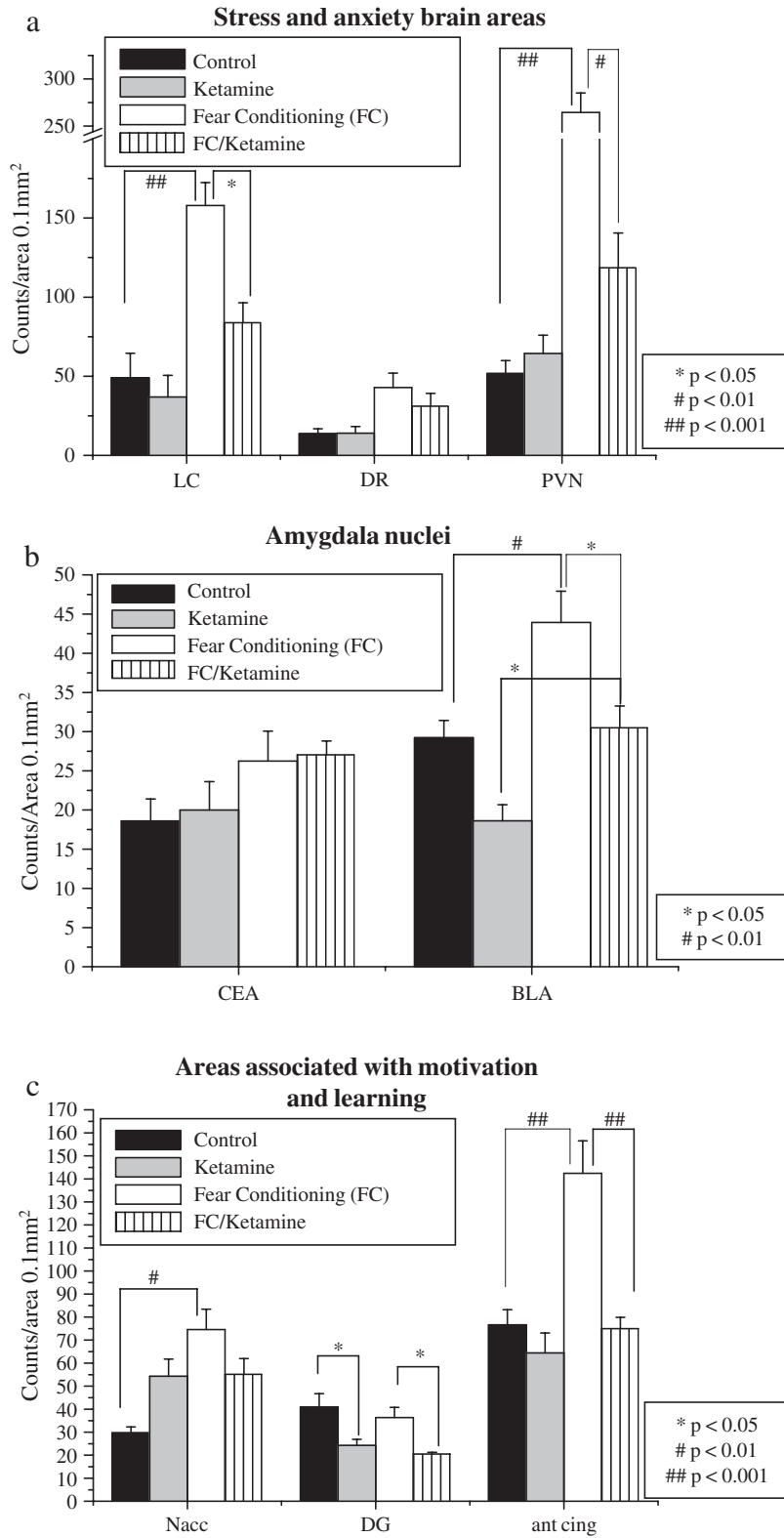
Fig. 3. cFos expression in brain areas. Significant differences shown on the graphs are according to Tukey's post hoc test. Significant differences between non-related groups (i.e. no fear conditioning and saline vs. fear conditioned and ketamine) are not shown. Fear conditioning increased cFos expression in (a) the locus coeruleus ($p<0.001$), paraventricular nucleus ($p<0.001$), (b) basolateral amygdala ($p=0.008$), (c) nucleus accumbens ($p=0.002$) and anterior cingulate ($p<0.001$). Ketamine itself decreased cFos expression in (c) the dentate gyrus in the presence of saline ($p=0.031$) and fear conditioning ($p=0.043$). A similar trend was seen in (b) the basolateral nucleus of the amygdala ($p=0.051$), which disappeared in the presence of fear conditioning ($p=0.026$). Ketamine significantly reversed the effect of fear conditioning in (a) the locus coeruleus ($p=0.016$), paraventricular nucleus ($p=0.009$), (b) basolateral amygdala ($p=0.015$), and (c) anterior cingulate ($p<0.001$). (DR = dorsal raphe, LC = locus coeruleus, PVN = paraventricular nucleus, CEA = central nucleus amygdala, BLA = basolateral nucleus amygdala, antcing = anterior cingulate, DG = dentate gyrus, Nacc = nucleus accumbens).

4. Discussion

4.1. Behavioural and neural correlates of stress

The main aim of this study was to examine how the influence of systemic ketamine administration manifests itself in neural and behavioural assays of fear-conditioned stress in rats. Fear conditioning was successful in eliciting stress, as witnessed by the increased (decreased) freezing (grooming) behaviour (Fig. 2) up to 30 min after the last conditioned tone. Consistent with our behavioural observations, the locus coeruleus, the dorsal raphe and the paraventricular nucleus, all areas involved in either stress regulation or associated with anxiety (Nash and Maickel, 1988; Chaouloff, 2000; Dunn et al., 2004) showed increased levels of cFos expression induced by fear-conditioned

stress (Fig. 3). In relation to our central hypothesis, administration of ketamine to fear-conditioned rats normalised stress-related behaviours (Fig. 2) and cFos levels in the above-mentioned brain areas, except for the dorsal raphe (Fig. 3). Thus, both the neural and behavioural data suggest that our fear-conditioning paradigm was effective in eliciting a stressful state and that ketamine was successful in normalising this state.

4.2. Differential activation in amygdala nuclei

As indicated in the Introduction, the amygdala performs a key role in the learning and expression of fear (LeDoux, 1992, 1998; Phillips and LeDoux, 1992; Walker and Davis, 2002). To this end, we found considerable fear-elicited cFos expression in the basolateral amygdala, but found only a small increase in
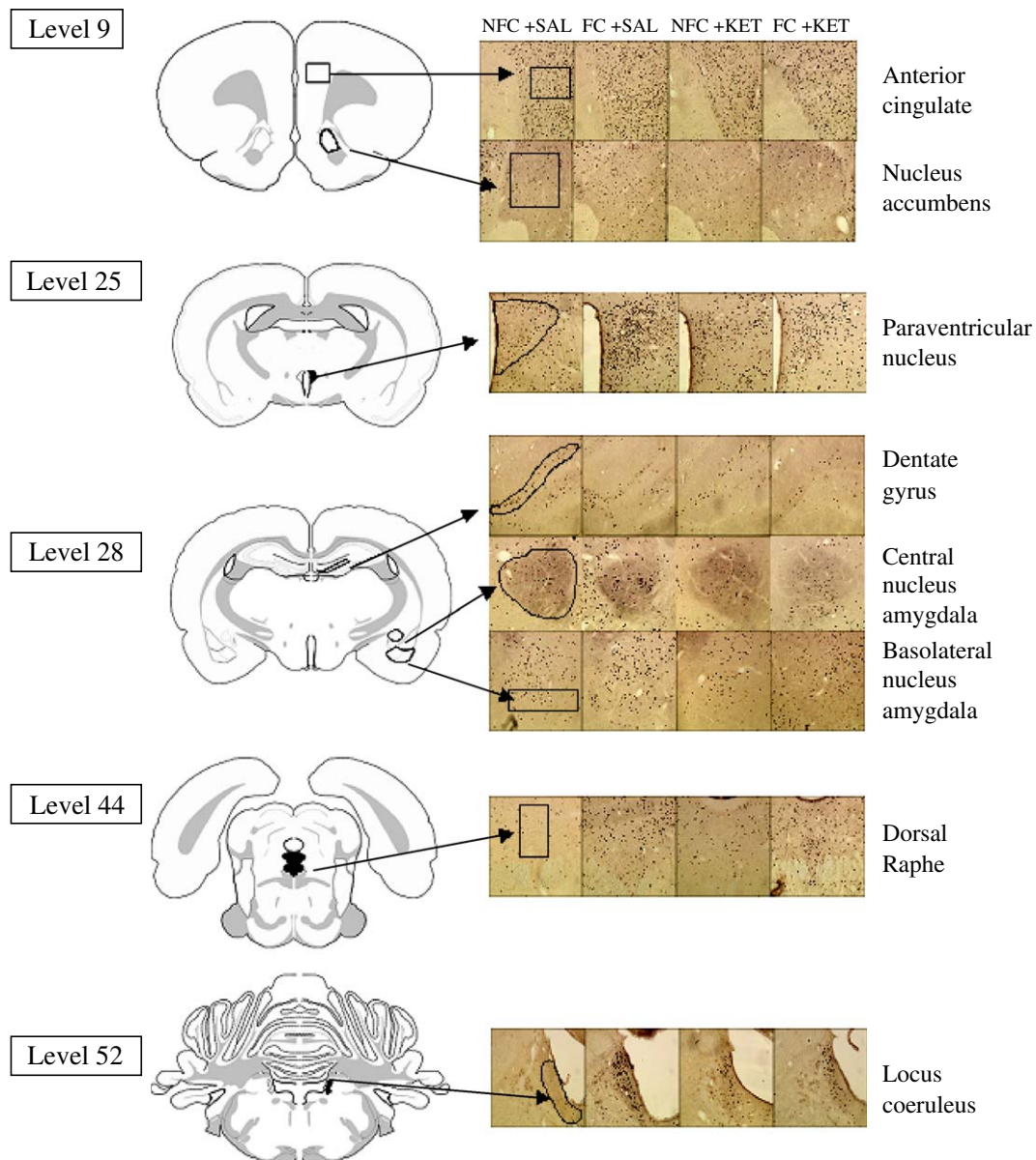

Fig. 4. cFos expression. Typical examples of the brain areas stained for cFos expression, visually showing the effects of the various treatments. Delineated areas depict actual areas measured. Brain slice levels were taken from the Swanson rat brain atlas (1992), with appropriate co-ordinates listed in Table 1.

cFos expression in the central nucleus (the increase was significant only after combining data from ketamine and non-ketamine groups). Whereas administration of ketamine normalised cFos expression in the basolateral nucleus, we found no such effect in the central nucleus. Interestingly, ketamine administration in the absence of fear conditioning decreased cFos expression slightly in the basolateral nucleus (although the effect was not significant) but not in the central nucleus.

The differential activation of the basolateral and central nuclei in response to fear conditioning and ketamine administration likely reflects the different functional roles performed by these areas (Cardinal et al., 2002). Goosens and Maren (2003), for example, have shown that that infusion of the NMDA antagonist D, L-2-amino-5-phosphonovalerate (APV) into either the basolateral or central nuclei blocks the acquisition of conditional fear. Residual fear memory is, however, retained following APV administration into the central nucleus, but not the basolateral, indicating that the latter nucleus is more critical to fear learning and memory. Koo et al. (2004) showed that the fibres that run through the central nucleus from the basolateral nucleus, and not the neurons within the central nucleus itself, are involved in fear conditioning. Our data also suggest that the basolateral amygdala plays the more important role in fear conditioning, since we found no effect of conditioning or ketamine in the central nucleus. Although our paradigm does not permit us to say whether ketamine blocked the acquisition or the expression of fear, previous experiments involving focal infusion of NMDA receptor antagonists (AP5 (2-amino-5-phosphonopentanoic acid) and APV) into the amygdala are consistent with the impairment of fear learning rather than expression (Fanselow and Kim, 1994; Schauz and Koch, 2000; Goosens and Maren, 2003). In summary, our data is consistent with the idea that ketamine-induced glutamatergic hypofunction impairs processes related to fear conditioning in the basolateral amygdala (Fanselow and Kim, 1994).

4.3. Other brain areas implicated in fear processing — the anterior cingulate and nucleus accumbens

The rat anterior cingulate has previously been shown to be involved in associative learning (Cardinal et al., 2002), particularly fear conditioning (Frankland et al., 2004; Gao et al., 2004). In our study, fear conditioning was associated with a marked increase in cFos expression in this area, with the increase being more than double the amount of cFos elicited in the control condition (Fig. 3). Administration of ketamine completely abolished the fear-related cFos expression, consistent with the tight bi-directional anatomical and functional linkage between the anterior cingulate and basolateral nucleus of the amygdala (Cardinal et al., 2002). The nucleus accumbens, an area primarily involved in motivation (Reynolds and Berridge, 2003; Salamone et al., 2005), also forms an integral part of this circuit (Levita et al., 2002), as it receives projections from both the anterior cingulate and the basolateral nucleus (Cardinal et al., 2002). We also observed an increase in fear-related cFos expression in the nucleus accumbens (Fig. 3). Ketamine administration did not, however, drive cFos expres-

sion back down to the level observed in the control group. Interestingly, ketamine alone elicited a slight, but non-significant, increase in cFos expression.

4.4. Failure to detect fear-induced activation in the dentate gyrus

We failed to find fear-related cFos expression in the dentate gyrus of the hippocampal formation. Indeed, ketamine administration was associated with slight decreases in cFos expression, below the level of the control group, in both the presence and absence of fear conditioning. The absence of fear-related cFos activity might be explained by the notion that the hippocampus plays a role in contextual fear memory but not in the type of tone-dependent fear conditioning (Thiels and Klann, 2002) used in our study. Perhaps other hippocampal regions, such as CA3, may have manifested fear-dependent cFos activity, although the main focus of our study was not the hippocampus.

4.5. Ketamine's confounding properties

Could the effects we observed be due to the anaesthetic properties of ketamine? Importantly, we used a sub-anaesthetic dose of ketamine that has previously been tested for its soporific actions upon rats' locomotor activity (Imre et al., 2006): Locomotor activity normalised by the 30 min mark at which we began fear conditioning in the present study. A study by Pallares et al. (1995) also indicated that a 12 mg/kg ketamine dose injected half an hour before testing did not interfere with locomotor activity. The authors concluded that the deficits encountered with the operant behavioural learning paradigm in their study could therefore not be due to the anaesthetic properties of the drug.

As ketamine is known to cause perceptual distortions, is it also possible that the drug prevented proper sensory encoding of the conditioned stimulus rather than blocking fear processing? de Bruin et al. (1999) showed that a 10 mg dose of ketamine did not alter auditory evoked potentials in a double-click paradigm in rats. This result suggests that conditioned-stimulus encoding was likely to be intact at the 16 mg dose used in our study, although it does not completely rule out the possibility of an analgesic effect. A study using an NMDA antagonist, AP5, infused into the amygdala, reported that reactions to footshocks between controls and AP5 rats were indistinguishable, even at a dose 4-fold higher than that required to impair learning (Miserendino et al., 1990; see also Campeau et al., 1992). These authors concluded that AP5 treatment did not have an analgesic effect but rather disrupted the association of the unconditioned and conditioned stimuli (Walker and Davis, 2002). In our study, we administered ketamine systemically, rather than locally. Yet our qualitative behavioural observations during conditioning suggest that ketamine-treated and control rats responded in the same fashion (e.g. jumping height during the shock appeared the same). The putative analgesic effect of ketamine is therefore unlikely to have played a significant role in our study.

4.6. Relevance for psychiatric disorders

In related work in our lab, we have demonstrated that administration of atypical, but not typical, neuroleptics partly restores the impaired neural and behavioural conditioned-fear responses introduced by ketamine (Pietersen et al., in preparation). We therefore speculate that the combination of ketamine administration and fear conditioning may in future provide an animal model for the emotional-processing deficits seen in schizophrenia (Paradiso et al., 2003; Sachs et al., 2004; Takahashi et al., 2004). It may, however, also prove valuable in the modelling of other psychiatric disorders featuring abnormal fear processing such as anxiety disorders (Doronbekov et al., 2005; Swanson et al., 2005).

5. Conclusion

The authors conclude that the administration of the glutamate antagonist ketamine blocks the expression of fear-conditioned stress in rats at multiple neural sites and at the behavioural level. We further suggest that the combination of fear conditioning and ketamine may provide an effective model in linking the breakdown of fear processing to hypoglutamatergic states. In particular, our work could have implications for disorders in which fear processing is abnormal, such as schizophrenia (Tsai and Coyle, 2002; Coyle and Tsai, 2004).

Acknowledgements

References

Campeau S, Miserendino MJ, Davis M. Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. Behav Neurosci 1992;106:569–74.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 2002;26:321–52.

Chaouloff F. Serotonin, stress and corticoids. J Psychopharmacol 2000;14:139–51.

Coyle JT, Tsai G. The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. Psychopharmacology (Berl) 2004;174:32–8.

Daniels WM, Pietersen CY, Carstens ME, Stein DJ. Maternal separation in rats leads to anxiety-like behavior and a blunted ACTH response and altered neurotransmitter levels in response to a subsequent stressor. Metab Brain Dis 2004;19: 3–14.

Davidson RJ, Irwin W. The functional neuroanatomy of emotion and affective style. Trends Cogn Sci 1999;3:11–21.

Davis M, Rainnie D, Cassell M. Neurotransmission in the rat amygdala related to fear and anxiety. Trends Neurosci 1994;17:208–14.

de Bruin NM, Ellenbroek BA, Cools AR, Coenen AM, van Luijtelaar EL. Differential effects of ketamine on gating of auditory evoked potentials and prepulse inhibition in rats. Psychopharmacology (Berl) 1999;142: 9–17.

Doronbekov TK, Tokunaga H, Ikejiri Y, Kazui H, Hatta N, Masaki Y, et al. Neural basis of fear conditioning induced by video clip: positron emission tomography study. Psychiatry Clin Neurosci 2005;59:155–62.

Dunn AJ, Swiergiel AH, Palamarchouk V. Brain circuits involved in corticotropin-releasing factor-norepinephrine interactions during stress. Ann N Y Acad Sci 2004;1018:25–34.

Fanselow MS, Kim JJ. Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. Behav Neurosci 1994;108:210–2.

Frankland PW, Bontempi B, Talton LE, Kaczmarek L, Silva AJ. The involvement of the anterior cingulate cortex in remote contextual fear memory. Science 2004;304:881–3.

Gao YJ, Ren WH, Zhang YQ, Zhao ZQ. Contributions of the anterior cingulate cortex and amygdala to pain- and fear-conditioned place avoidance in rats. Pain 2004;110:343–53.

Goosens KA, Maren S. Pretraining NMDA receptor blockade in the basolateral complex, but not the central nucleus, of the amygdala prevents savings of conditional fear. Behav Neurosci 2003;117:738–50.

Imre G, Fokkema DS, Boer JA, Ter Horst GJ. Dose-response characteristics of ketamine effect on locomotion, cognitive function and central neuronal activity. Brain Res Bull 2006;69(3):338–45.

Kim M, McGaugh JL. Effects of intra-amygdala injections of NMDA receptor antagonists on acquisition and retention of inhibitory avoidance. Brain Res 1992;585:35–48.

Koo JW, Han JS, Kim JJ. Selective neurotoxic lesions of basolateral and central nuclei of the amygdala produce differential effects on fear conditioning. J Neurosci 2004;24:7654–62.

LeDoux JE. Brain mechanisms of emotion and emotional learning. Curr Opin Neurobiol 1992;2:191–7.

LeDoux J. Fear and the brain: where have we been, and where are we going? Biol Psychiatry 1998;44:1229–38.

LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci 2000;23:155–84.

Lee HJ, Choi JS, Brown TH, Kim JJ. Amygdalar nmda receptors are critical for the expression of multiple conditioned fear responses. J Neurosci 2001;21:4116–24.

Levita L, Dalley JW, Robbins TW. Nucleus accumbens dopamine and learned fear revisited: a review and some new findings. Behav Brain Res 2002;137:115–27.

Li XF, Phillips R, LeDoux JE. NMDA and non-NMDA receptors contribute to synaptic transmission between the medial geniculate body and the lateral nucleus of the amygdala. Exp Brain Res 1995;105: 87–100.

Maren S. Neurobiology of Pavlovian fear conditioning. Annu Rev Neurosci 2001;24:897–931.

Maren S, Fanselow MS. The amygdala and fear conditioning: has the nut been cracked? Neuron 1996;16:237–40.

Miserendino MJ, Sananes CB, Melia KR, Davis M. Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. Nature 1990;345:716–8.

Morrow BA, Elsworth JD, Roth RH. Fear-like biochemical and behavioral responses in rats to the predator odor, TMT, are dependent on the exposure environment. Synapse 2002;46:11–8.

Nash Jr JF, Maickel RP. The role of the hypothalamic–pituitary–adrenocortical axis in post-stress induced ethanol consumption by rats. Prog Neuropsychopharmacol Biol Psychiatry 1988;12:653–71.

Pallares MA, Nadal RA, Silvestre JS, Ferre NS. Effects of ketamine, a noncompetitive NMDA antagonist, on the acquisition of the lever-press response in rats. Physiol Behav 1995;57:389–92.

Paradiso S, Andreasen NC, Crespo-Facorro B, O'Leary DS, Watkins GL, Boles Ponto LL, et al. Emotions in unmedicated patients with schizophrenia during evaluation with positron emission tomography. Am J Psychiatry 2003;160:1775–83.

Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 1992;106:274–85.

Pietersen CY, Bosker FJ, Postema F, den Boer JA. Fear conditioning and shock intensity: the choice between minimizing the stress induced and reducing the number of animals used. Lab Anim 2006;40:180–5.

Reynolds SM, Berridge KC. Glutamate motivational ensembles in nucleus accumbens: rostrocaudal shell gradients of fear and feeding. Eur J Neurosci 2003;17:2187–200.

Sachs G, Steger-Wuchse D, Kryspin-Exner I, Gur RC, Katschnig H. Facial recognition deficits and cognition in schizophrenia. Schizophr Res 2004;68:27–35.

Salamone JD, Correa M, Mingote SM, Weber SM. Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. Curr Opin Pharmacol 2005;5:34–41.

Schauz C, Koch M. Blockade of NMDA receptors in the amygdala prevents latent inhibition of fear-conditioning. Learn Mem 2000;7:393–9.

Sharp JL, Zammit TG, Azar TA, Lawson DM. Stress-like responses to common procedures in male rats housed alone or with other rats. Contemp Top Lab Anim Sci 2002;41: 8–14.

Suzuki T, Ishigooka J, Watanabe S, Miyaoka H. Enhancement of delayed release of dopamine in the amygdala induced by conditioned fear stress in methamphetamine-sensitized rats. Eur J Pharmacol 2002;435:59–65.

Swanson LW. Brain maps: structure of the rat brain. Amsterdam: Elsevier Publishing Company; 1992.

Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat Rev Drug Discov 2005;4:131–44.

Takahashi H, Koeda M, Oda K, Matsuda T, Matsushima E, Matsuura M, et al. An fMRI study of differential neural response to affective pictures in schizophrenia. NeuroImage 2004;22:1247–54.

Tang J, Ko S, Ding HK, Qiu CS, Calejesan AA, Zhuo M. Pavlovian fear memory induced by activation in the anterior cingulate cortex. Mol Pain 2005;1:6.

Thiels E, Klann E. Hippocampal memory and plasticity in superoxide dismutase mutant mice. Physiol Behav 2002;77:601–5.

Tsai G, Coyle JT. Glutamatergic mechanisms in schizophrenia. Annu Rev Pharmacol Toxicol 2002;42:165–79.

Walker DL, Davis M. The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. Pharmacol Biochem Behav 2002;71:379–92.