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DECISION-MAKING PERFORMANCE IS RELATED TO LEVELS OF ANXIETY AND DIFFERENTIAL RECRUITMENT OF FRONTOSTRIATAL AREAS IN MALE RATS

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Abstract—In humans, high levels of anxiety are associated with poor performance in the Iowa Gambling Task (IGT). The IGT measures decision-making under conditions of uncertainty. In this study, we investigated the association between anxiety and decision-making in rats. Rats were screened for anxiety on the elevated plus maze (EPM) and subsequently tested in a rat analogue of the IGT (r-IGT). We explored the role of frontostriatal areas related to r-IGT performance using c-fos immunohistochemistry following the last training-session. High levels of anxiety were associated with poor r-IGT performance: high anxious rats made fewer choices for the advantageous option and collected fewer sucrose pellets in the r-IGT than low anxious rats. Analysis of win-stay/lose-shift behaviour of choices for the advantageous option revealed that good performing-low anxious subjects showed an increase in win-stays and a decrease in lose-shifts across trial blocks while poor performing-high anxious subjects did not. Furthermore, decision-making performance and, indirectly, anxiety levels were related to neural activity in parts of the medial prefrontal cortex, that is prelimbic and infralimbic cortex, and in parts of the striatum, that is nucleus accumbens shell and core. These data suggest a similar frontostriatal circuitry underlying affective decision-making in humans and rats. © 2011 Published by Elsevier Ltd on behalf of IBRO.

Key words: anxiety, decision-making, rats, c-fos, prefrontal cortex, striatum.

It is well-established that highly anxious individuals are at risk for mood disorders and drug addiction (Bennet and Stirling, 1998; Willinger et al., 2002; Mathews and MacLeod, 2005; National Institute of Mental Health, 2008). This may be related to altered generation and perception of emotions. However, anxiety is known to affect cognitive processes as well, for example by inducing a negative cognitive bias (Mathews, 1990; Mathews and Mackintosh, 1998; Barlow, 2002; Calvo et al., 2003; Bishop et al., 2004). Recently, we (de Visser et al., 2010a) and others (Miu et al., 2008) have shown that anxiety affects decision-making: highly anxious human subjects perform poorly in the Iowa Gambling Task (IGT). The IGT measures decision-making processes by simulating real-life decisions involving reward, punishment, and uncertainty of outcomes. In the IGT, healthy participants learn to prefer long-term advantageous options associated with immediate moderate rewards over long-term disadvantageous options associated with immediate high rewards (Bechara et al., 1994, 1999).

The neural underpinnings of the relationship between anxiety and decision-making remain elusive. However, a number of brain areas have been implicated in both anxiety and IGT-like decision-making, such as the medial prefrontal cortex (mPFC), dorsolateral prefrontal cortex (dlPFC), anterior cingulate cortex (ACC), ventral striatum (vSTR) and amygdala (AMY) (e.g. Bechara et al., 1999; Grachev and Apkarian, 2000; Ernst et al., 2002; Bolla et al., 2004; Etkin et al., 2004; Brand et al., 2006; Lopes et al., 2007; Da Cunha et al., 2008; Li et al., 2010; Salomons et al., 2010). We addressed the relationship between anxiety and decision-making in rats in the present study. For this purpose, male rats were first tested on a validated rodent test for anxiety, that is the elevated plus maze (Pellow et al., 1985). Subsequently their decision-making performance was assessed using a validated test for decision-making, that is the rodent version of the Iowa Gambling Task (r-IGT, van den Bos et al., 2006b; Homberg et al., 2008). While performance was measured as the number of advantageous choices and the number of obtained rewards, choice strategies were determined using win-stay/lose-shift analysis. We predicted that highly anxious rats would perform poorly on the r-IGT. To study the neural substrates underlying decision-making in relation to anxiety in rats, expression of the immediate early gene c-fos—marker for neural activity—was measured in brain areas suggested to be involved in both decision-making and anxiety in humans, that is parts of the prefrontal cortex, ventral striatum, and amygdala.

EXPERIMENTAL PROCEDURES

Subjects

Male Wistar rats (n=25), 10 weeks of age, were purchased from Harlan (Horst, the Netherlands). They were housed in pairs in macrolon type IV cages under a reversed 12 h light/dark cycle (lights off at 7 AM). A shelter and paper tissues were provided as

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Abbreviations: BSA, bovine serum albumin; Cg1, cingulate cortex; EPM, elevated plus maze; IGT, Iowa Gambling Task; mPFC, medial prefrontal cortex; NDS, normal donkey serum; OFC, orbitofrontal cortex; PBS, phosphate-buffered saline; r-IGT, rodent version of the Iowa Gambling Task.

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cage enrichment. Food and water were freely available except during testing (see below). Room temperature was controlled at 21±2 °C with a relative humidity of 60±15%. A radio provided background noise. All experiments were approved by the Animal Ethics Committee of Utrecht University and were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Elevated plus maze (EPM)

The EPM was made of grey PVC and elevated 75 cm above the floor. The four arms (50×10 cm^2) formed a cross with the central platform. A wall (height: 30 cm) of non-transparent material enclosed two arms, located opposite to each other. Each rat was placed on the central platform facing one of the enclosed arms and allowed to freely explore the maze for 5 min. In between trials, the maze was cleaned with warm water and dried thoroughly using clean towels. Behaviour was recorded on DVD and scored afterward using Observer 5.0 (Noldus Information Technology, Wageningen, The Netherlands). Behaviour on the EPM was analysed according to Rodgers and Johnson (1995). Spatiotemporal measures comprised the time spent on the open and closed arms of the maze, the percentage of time spent on the open arms (an arm entry was scored when the animals had at least three paws on the arm), the number of open arm entries, closed arm entries and total arm (open+closed) entries. Behavioural measures comprised frequency of head dips (a downward movement of head/shoulders over the side of the open arms), rearing (vertical movement while standing on two hind legs in the visible part of the maze), risk-assessment (animal is inside a closed arm with its head outside this closed arm) and end-arm exploration (animal is within one body-length from the outer edge of the open arm). Head dips and rearings were scored as either "unprotected," that is when performed on the open arm, or "protected" when performed on the closed arm or centre platform.

Rodent Iowa Gambling Task (r-IGT)

The same apparatus and procedure was used as previously described (van den Bos et al., 2006b; Homberg et al., 2008) with minor modifications. The r-IGT apparatus was made of wood and consisted of a start box, choice area and four arms. Before the start of testing, rats were habituated to the apparatus in a 10 min free exploration trial. Two days later, they were mildly food restricted (approximately 95% of free feeding body weight) and tested for a period of 10 days. Testing occurred during two 5-day periods. Food was freely available on weekend days. A trial started by lifting the slide door of the start box. The rat could freely enter the choice area of the apparatus and choose one of the four arms. The chosen arm was only closed when the rat had entered a choice arm with its full body, including its tail. At the end of the arm, rats could obtain sucrose pellets or quinine-treated sucrose pellets (baited arms; see below) or no pellets at all (empty arms). Each trial had a maximum duration of 6 min. The inter-trial interval was 30 s. The rats received a total of 120 trials across 10 days. Rewards were 45 mg sucrose pellets (Bioserve Inc, Frenchtown, NJ, USA) and punishments were quinine-treated sucrose pellets that were unpalatable but not uneatable. Most rats consumed the quinine-treated pellets once, but left them uneaten after briefly tasting them on subsequent encounters. Rats that consistently ate the quinine-treated sucrose pellets were excluded from the analysis. Of the four arms in the maze, two were baited and two were empty. The two empty arms were included to measure non-reward related exploration (van den Bos et al., 2006b; Homberg et al., 2008). The two baited arms consisted of a "bad" arm and a "good" arm. In the "bad" or long-term disadvantageous arm, the rats received occasional big rewards (three sucrose pellets in one out of 10 trials) among frequent punishments (three quinine-treated sucrose pellets in nine out of 10 trials). In the "good" or long-term advantageous arm, the rats received frequent small rewards (one sucrose pellet in eight out of 10 trials) and infrequent punishments (one quinine-treated sucrose pellet in two out of 10 trials). This provided the same principle as in the human IGT: an option with a chance of a big reward (three sucrose pellets), but with little long-term success (three sucrose pellets per 10 trials; cf. decks A and B; Bechara et al., 1994) and an option with a chance of a small reward (one sucrose pellet), but with higher long-term success (eight sucrose pellets per 10 trials; cf. decks C and D). The location of the baited and empty arms, as well as "good" and "bad" arms was counterbalanced across subjects.

To determine the choice behaviour of the rats, the main parameter of interest was the number of choices for the advantageous option as a fraction of total number of trials. This was expressed in blocks of 20 trials to study task progression. Scores in the last session (trial 106–120) were taken as a measure of final IGT performance of rats. The total number of sucrose pellets collected during the task (trial 1–120) was used as a measure of overall task performance to reflect the final "budget" (cf. monetary budget in the human IGT, van den Bos et al., 2006a). Furthermore, the number of switches between different arms was calculated as a measure of exploratory behaviour. This was expressed per block of 20 trials to study the change in exploratory behaviour across the task. Win-stay/lose-shift behaviour was measured after encounters with quinine or sucrose in the advantageous arm. Thus, when rats encountered a sucrose reward, their subsequent choice was scored as a win-stay when they revisited the advantageous arm, otherwise as a win-shift. When rats encountered a quinine punishment, their subsequent choice was scored as a lose-shift when the rat switched to another arm, otherwise as a lose-stay. Win-stay and lose-shift were calculated as a fraction of the total number of encounters with sugar (win) and quinine (loss) respectively. Thus, an increase in win-stay behaviour was paralleled by a decrease in win-shift behaviour. Similarly, a decrease in lose-shift behaviour was paralleled by an increase in lose-stay behaviour.

Procedure

After arrival, rats were allowed to habituate to the housing conditions in the animal facility for 2–3 weeks. Cages were cleaned once a week. Rats were handled two to three times a week to familiarize them with the experimenters. After the habituation period, rats were first tested on the EPM. One week later, rats were tested in the r-IGT for two weeks under mild food restriction. All experiments were carried out during the dark phase of the day–night cycle, between 8.30 AM and 5 PM.

c-Fos immunohistochemistry

c-Fos immunostaining was performed for analysis of neural activity in brain areas involved in r-IGT performance. Two hours after the last session in the r-IGT (15 trials; trial 106–120), the animals were decapitated. Brains were quickly removed and frozen in liquid (−80 °C) 2-methylbutane which was cooled with dry ice and stored at −80 °C. Coronal sections (20 μm) were cut on a cryostat (Leica CM3050S) and mounted on Starfrost adhesive slides (Knittel Glaser, Waldemar Knittel, Germany) and stored at −20 °C. For the immuno-histochemical detection of c-fos, rabbit anti-c-fos (Calbiochem, Darmstadt, Germany) was used. During the staining procedure the sections were rinsed several times after every step in 0.01 M phosphate-buffered saline (PBS; pH 7.4). First, the sections were dehydrated. Endogenous peroxidase was blocked by treatment with H_2O_2 (0.1%) for 30 min. Sections were pre-incubated with 5% normal donkey serum (NDS) and 1% bovine serum albumin (BSA) in PBS (PBS-BSA 1%+NDS 5%) for 30 min before the rabbit anti-c-fos incubation (1:4000 in PBS-BSA 1%+NDS 5%, 4 °C, 24 h). Negative controls were incubated with the PBS-BSA 1%+NDS 5% solution. Next, the sections were

incubated with donkey–anti-rabbit IgG Biotin SP conjugate (1:400 in PBS-BSA 1%+NDS 5%, Jackson ImmunoResearch Laboratories, Inc., PA, USA) for 45 min. Subsequently, the sections were incubated with avidin-horseradish peroxidase solution (1:400 in PBS-BSA 1%+NDS 5% VECTASTAIN® ELITE ABC, Brunswich Chemie, Amsterdam, The Netherlands) for 60 min. Then, slices were pre-incubated with inactive diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, St. Louis, MO, USA) solution containing nickel sulfate. To activate DAB for visualization of bound peroxidase complexes, the substrate H_2O_2 (30%, 1:2000) was added to the DAB solution and incubated for 5 min. Afterward the sections were dehydrated in alcohol and coverslipped.

The images of brain sections were projected (10× magnification) and digitized using an Olympus BX 51 microscope (Olympus, Tokyo, Japan) with a high-resolution digital camera interfaced with a computer. The anatomical localization was aided by use of adjacent Nissl stained sections and illustrations in a stereotaxic atlas (Paxinos and Watson, 2005). The following subregions of the prefrontal cortex were investigated: orbitofrontal cortex (OFC; +4.20 from Bregma), insular cortex (+1.92 from Bregma), cingulate cortex (Cg1; 2.52 from Bregma) and medial prefrontal cortex, that is prelimbic (PrL; 2.52 from Bregma) and infralimbic cortex (IL; 2.52 from Bregma). For the ventral striatum both the nucleus accumbens core (NaC; +1.92 from Bregma) and shell (NaS; +1.92 from Bregma) were analyzed and the amygdala included the basolateral part (BLA; −1.72 from Bregma) and central nucleus (CeN; −1.72 from Bregma). For each region at least two overt landmarks were used. For quantitative analysis of c-fos positive cells, the program Leica QWIN (image processing and analysis software, Cambridge, UK) was used. Left and right hemispheres were separately analyzed in one section. The number of positive cells was then averaged for each animal and expressed per 1.0 mm^2.

Statistical analysis

All statistical analyses were carried out using SPSS 16.0 for Windows.

As the main measure for anxiety, the percentage of time spent on the open arms was used. This parameter is pharmacologically validated by anxiolytic/anxiogenic compounds (Pellow et al., 1985; Cruz et al., 1994; Violle et al., 2009). Furthermore, open arm time is commonly used as a parameter to classify rats into different levels of anxiety (Pawlak et al., 2008) or for selective inbreeding experiments (e.g. HAB/LAB rats, Landgraf and Wigger, 2002). To study the relationship between anxiety on the EPM and decision-making, we calculated Pearson correlations for the open arm time and closed arm entries on the EPM and parameters of the r-IGT (see Results section for details). Since the measurement of additional ethologically-derived parameters has been shown to increase the sensitivity of the EPM (Rodgers and Johnson, 1995; Carobrez and Bertoglio, 2005), behavioural measures were included in the present study and related to r-IGT performance. Statistical details are mentioned in the Results section where appropriate.

As brains were removed 2 h after the last r-IGT session, the number of c-fos positive cells can only be directly related to performance during trial block 106–120. To test whether final r-IGT performance was related to neural activity, animals were grouped using a split-median approach based on their performance as reflected by the number of advantageous choices. Animals with an r-IGT score (trial 106–120) above the median were classified as "good" performers, animals with an r-IGT score below the median (trial 106–120) were classified as "poor performers." Subsequently, differences in the number of c-fos positive cells between "good" and "poor" performers were tested using independent *t*-tests or non-parametric Mann–Whitney *U*-tests when data were not normally distributed. Furthermore, to investigate whether differences existed in the task progression between "good" and "poor" performers, performance as well as the number of switches between arms were calculated in blocks of 20 trials and within/between-group differences were tested using a repeated measures ANOVA (within-subjects factor: trial block; between-subjects factor: performance group). Greenhouse-Geisser correction was performed whenever sphericity was violated. Win-stay/lose-shift behaviour in "good" and "poor" performers was calculated and tested in blocks of 40 trials because of the relatively low number of occurrences of losses. Differences between "good" and "poor" performers regarding levels of anxiety were tested using independent *t*-tests to ensure that the relationship between anxiety and decision-making found with correlational analysis was also present when using split-median approach for r-IGT performance.

Statistical significance was set at $P \leq 0.05$ (two-tailed); NS: non-significant. Unless otherwise indicated, all reported values are means±SEMs.

RESULTS

Anxiety and r-IGT performance: correlations

Anxiety affected r-IGT performance reflected by a significant positive correlation between the time spent on the open arms of the EPM and the fraction of advantageous choices during the final r-IGT session ($r=0.407$, $P=0.021$) and the total number of sugar pellets obtained ($r=0.536$, $P=0.002$). Thus, higher levels of anxiety were correlated with poorer r-IGT performance (see also Fig. 1) and lower pay-off during the task in terms of obtained reward. Notably, there was no correlation between the number of closed arm entries on the EPM, a measure for general activity, and r-IGT performance (advantageous choices: $r=-0.077$, $P=0.676$; sugar pellets: $r=0.139$, $P=0.448$), indicating that the relationship between EPM and r-IGT is specific for the anxiety-related component of the EPM.

Behavioural measures such as rearings and head dips contribute to the variance in EPM behaviour independently of spatiotemporal measures such as open arm time (Rodgers and Johnson, 1995). This prompted us to investigate the relationship between behavioural EPM measures and r-IGT performance in more detail. Correlation analysis showed a significant negative relationship between protected head dips and the fraction of advantageous arm choices during the last 15 trials of the r-IGT ($r=-.438$, $n=32$, $P=0.012$), thus indicating that a high number of protected head dips was related to a poor decision-making performance. None of the other behavioural measures showed any significant correlations with r-IGT performance (all parameters, $P>0.10$).

Split median for performance

Rats classified as "good" performers (split-median approach, see Methods) showed an average score of 0.87±0.03 choices for the advantageous option during the last session vs. an average of 0.50±0.04 advantageous choices for animals classified as "poor" performers. Note that both groups showed an overall preference for the advantageous option over the disadvantageous or empty options (chance level for the advantageous option is 0.25). In line with results presented above, "good" performers in the r-IGT displayed lower levels of anxiety in the EPM,

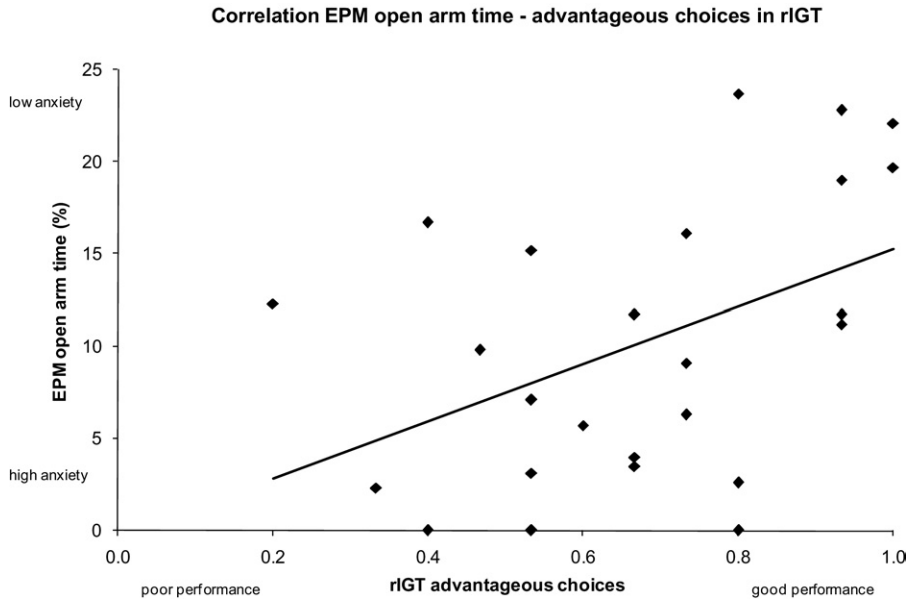

Fig. 1. Correlation between r-IGT performance and open arm time on the EPM. See text for statistical details.

reflected by a higher percentage of time spent on the open arm compared to "poor" performers (13.7±2.4% of open arm time vs. 7.0±1.6% of open arm time, $t=2.408$, $df=23$, $P=0.024$). To determine differences in the task progression between "good & non-anxious" performers and "poor & anxious" performers, performance level was calculated across trial blocks (Fig. 2A). There was a significant trial block×performance group interaction ($F(5,115)=5.052$, $P<0.001$), indicating that "good" performers showed a steeper increase in the fraction of advantageous choices across trials than "poor" performers. In fact in "poor" performers, the performance levelled off during the last 40 trials of the task. The number of switches (Fig. 2B) were clearly different between "good" and "poor" performers: "good" performers showed a gradual decrease in the number of switches, while this was absent in "poor" performers (trial block×performance group: $F(5,115)=5.225$, $P=0.002$).

Analysis of win-stay/lose-shift behaviour revealed different choice strategies in "good" and "poor" performers (Fig. 2C, D): while "good" performers showed an increase in win-stays and a decrease in lose-shifts across trial blocks (win-stay, trial block: $F(2,22)=11.446$, $P<0.001$; lose-shift, trial block: $F(2,22)=3.988$, $P=0.033$), "poor" performers hardly showed changes in these parameters across trial blocks (win-stay, trial block: $F(2,24)=3.261$, $P=0.056$, NS; lose-shift, trial block: $F(2,24)=0.173$, $P=0.842$, NS).

Overall the data show, that "good" and "poor" performers do not differ in their behaviour in the early trial blocks of the task, but clearly do so in last trial blocks.

C-fos immunohistochemistry

C-Fos expression in "good" and "poor" performers is presented in Fig. 4, while Fig. 3 depicts schematic diagrams of areas in which c-fos expression was quantified. The number of positive cells was higher in the prelimbic cortex ($t=-2.555$, $df=20$, $P=0.019$), infralimbic cortex ($t=-2.387$, $df=19$, $P=0.028$) and nucleus accumbens shell ($t=-3.941$, $df=19$, $P<0.001$) in "poor" performers compared to "good" performers. In contrast, c-fos expression in the nucleus accumbens core was higher in "good" performers compared to "poor" performers ($t=-2.300$, $df=16$, $P=0.035$). No differences were observed between "good" and "poor" performers in orbitofrontal cortex, insular cortex, cingulate area 1, basolateral amygdala and central nucleus of the amygdala (all areas $P>0.10$).

Further correlational analysis revealed a negative correlation between c-fos expression in the nucleus accumbens shell and r-IGT performance during the last 15 trials ($r=-.514$, $P=0.017$) as well as open arm time in the EPM ($r=-.454$, $P=0.038$). Thus, high activity in the nucleus accumbens shell was associated with poor performance in the r-IGT and high levels of anxiety in the EPM.

DISCUSSION

The present study yielded two main findings: (1) anxiety profoundly affects decision-making performance in the r-IGT in male rats; (2) levels of c-fos expression in medial prefrontal cortex and ventral striatum differentiate between "good" performing, non-anxious subjects and "poor" performing, anxious subjects suggesting the involvement of a frontostriatal circuitry in the interaction between anxiety and decision-making.

Relationship between anxiety and decision-making in rats

In line with studies in humans (Miu et al., 2008; de Visser et al., 2010a) high anxiety levels, as defined by the time spent on the open arms of the EPM, were associated with poor IGT-performance in rats. The only other EPM param-

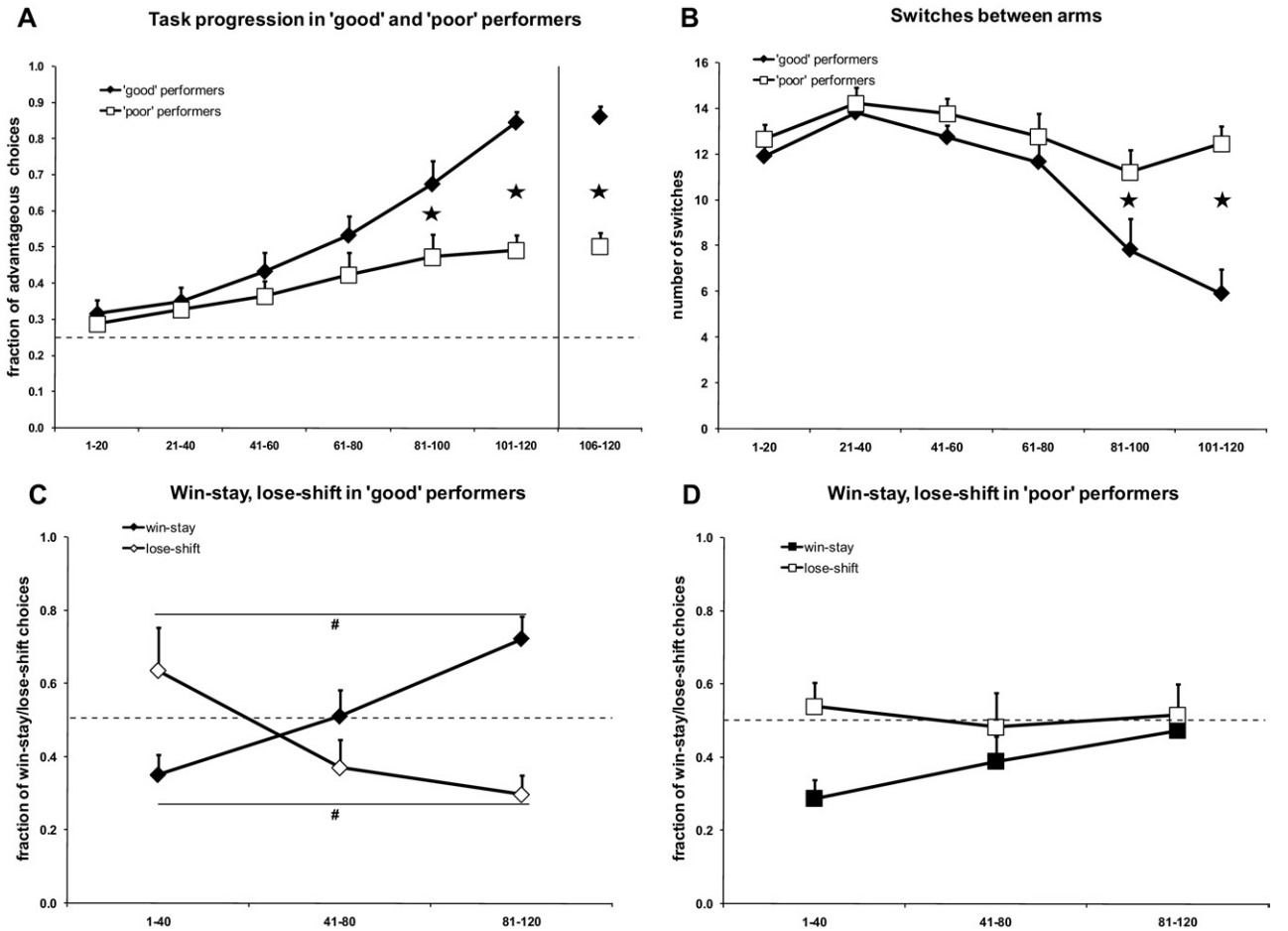

Fig. 2. (A) Task progression reflected as the mean (+SEM) fraction of visits to advantageous arms to the total visits per block of 20 trials for "good" and "poor" performers. (B) Mean (+SEM) number of switches between arms for "good" and "poor" performers per block of 20 trials (maximum number per trial block=19). (C) Win-stay/lose-shift choices in the advantageous arms for "good" performers. Shown are mean (+SEM) fractions of win-stays and lose-shifts as fraction of the total number of encounters with sucrose pellets (win) and quinine-saturated sucrose pellets (loss) respectively per block of 40 trials. (D) Win-stay/lose-shift choices in the advantageous arm for "poor" performers. Shown are mean (+SEM) fractions of win-stays and lose-shifts as fraction of the total number of encounters with sucrose pellets (win) and quinine-saturated sucrose pellets (loss) respectively per block of 40 trials. * $P<0.05$ between groups; # significant within-subjects effect of trial block (see text for statistical details).

eter related to r-IGT performance was the number of protected head dips, which correlated negatively with r-IGT performance. Head dipping behaviour is suggested to be an expression of risk-assessment associated with increased levels of anxiety (Lopes et al., 2007). Therefore the relationship between protected head dips and r-IGT performance supports the interpretation that high anxiety on the EPM is associated with poor performance on the r-IGT. We did not observe a relationship between closed arms entries, a measure of activity, and r-IGT performance indicating that the effect on decision-making was specific for anxiety-related parameters in the EPM. In the present rat study, we did not observe the U-shaped relationship between anxiety and IGT performance previously observed in men, that is a poorer performance in both low and highly anxious subjects compared to medium anxious subjects (de Visser et al., 2010a). An explanation for this discrepancy may be the relative high levels of anxiety in our rats compared to other reports in Wistar rats (Henniger et al., 2000; Jones et al., 2008; Ludwig et al., 2008). Consequently, the levels of anxiety of our low anxious animals are relatively high so that we may not have had truly non-anxious animals. Interestingly, poor decision-makers in a different r-IGT paradigm did show increased risk-taking behaviour on the EPM (Rivalan et al., 2009). This is reminiscent of the risky decision-making observed in low anxiety men (de Visser et al., 2010).

Anxiety may affect cognitive functioning by a number of mechanisms. For example, high anxiety is associated with negative cognitive bias and increased risk avoidance in humans (Mathews, 1990; Mathews and Mackintosh, 1998; Barlow, 2002; Bishop et al., 2004; Calvo et al., 2003; Maner and Schmidt, 2006). According to this interpretation, highly anxious subjects should avoid the disadvantageous risky options in the IGT. However, both in humans and in rats, highly anxious subjects show an increased number of disadvantageous choices (present study; Miu et al., 2008; de Visser et al., 2010a). Alternatively, anxiety may alter the perception of wins and losses in the advantageous and disadvantageous options. Both the human

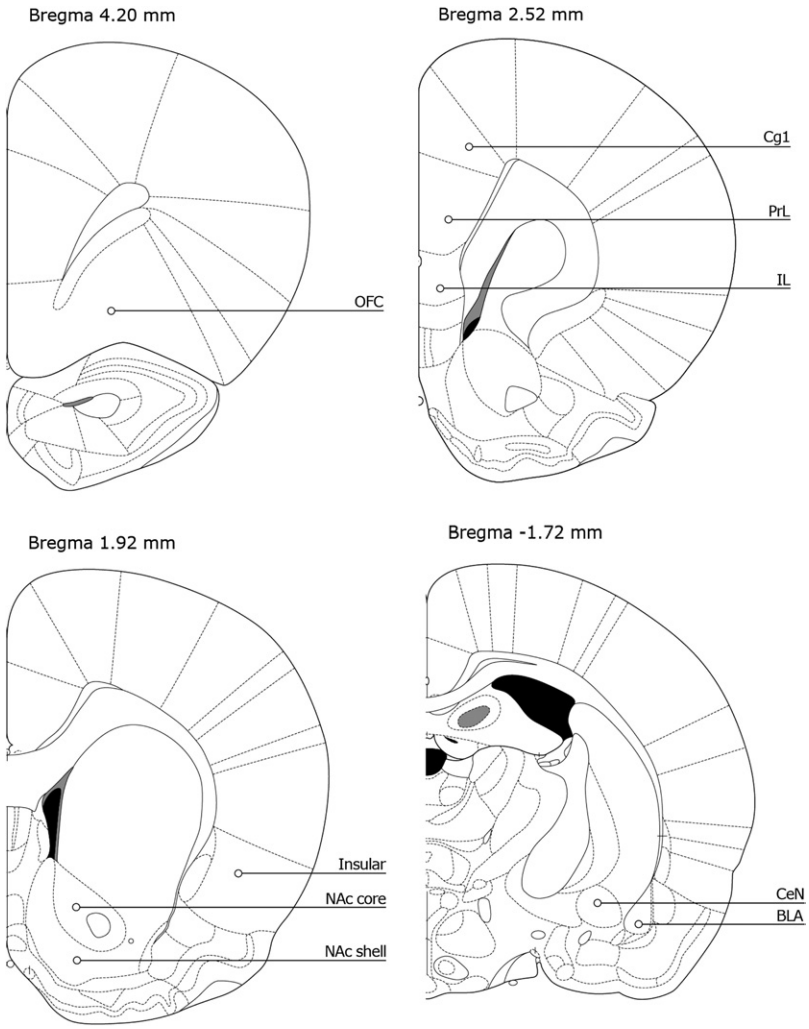

Fig. 3. Schematic representation of brain areas used to determine neural activity by c-fos immunohistochemistry (based on Paxinos and Watson, 2005). OFC, orbitofrontal cortex; Cg1, cingulate area 1; PrL, prelimbic cortex; IL, infralimbic cortex; INS, insular cortex; NaS, nucleus accumbens shell; NaC, nucleus accumbens core; CeN, central nucleus of the amygdala; BLA, basolateral amygdala. Position of coronal section relative to bregma is noted.

and rodent IGT are characterized by ambiguity (Brand et al., 2006). The outcome of each choice is uncertain, and in both advantageous and disadvantageous options individuals repeatedly encounter losses. To ensure optimal performance, it is crucial that over time subjects suppress the tendency to respond to the moderate losses in the advantageous option and instead learn to recognize this as the better long-term option despite the occasional losses. Our analysis of win-stay/lose-shift behaviour suggests that high anxiety subjects may respond hypersensitive to the ambiguous conditions and repeated encounters with losses in the advantageous option with enhanced lose-shift behaviour resulting in a less optimal decision-making strategy, that is a weaker cognitive control over immediate losses in the process of developing a long-term strategy. The inability of high anxiety subjects to suppress their responding to losses in the advantageous arm in the r-IGT may in fact be reminiscent of the finding that rats selectively bred for high anxiety showed impaired extinction of learned fear compared to their low anxiety counterparts (Muigg et al., 2008). The choice strategy of high anxiety subjects is further characterized by continued exploratory behaviour, which is reflected by the relatively high number of switches between arms compared to low anxiety subjects. The latter show an attenuation of lose-shift behaviour as well as switching behaviour over time, indicating a more efficient transition from exploration in the first part of the task to learn contingencies to exploitation of the advantageous option in the second part of the task. Interestingly, while the enhanced lose-shift behaviour in high anxiety subjects suggests a changed perception of the losses encountered in the r-IGT, win-stay behaviour is decreased in high anxiety subjects compared to low anxiety subjects, suggesting an altered perception of wins as well. Although there is a tendency for an increase in win-stays across trial blocks in high anxiety subjects, the fraction of win-stay choices in the last part of the task is markedly lower compared to low anxiety subjects. Anxiety could interfere with the ability of

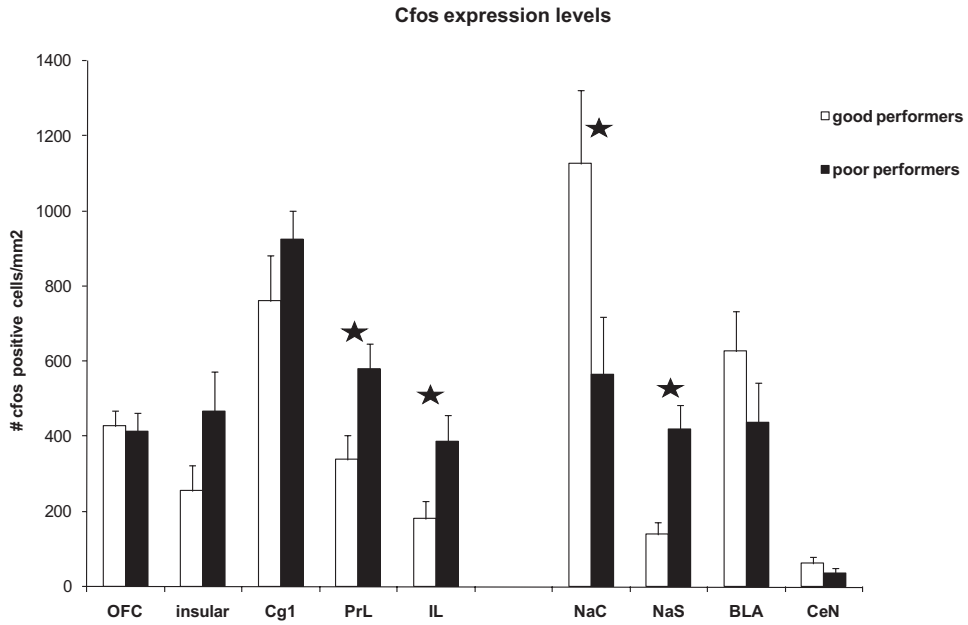

Fig. 4. Levels of c-fos expression (as the number of positive cells per mm^2) in different brain areas for rats classified as "good" and "poor" performers using a split-median approach based on the number of advantageous choices during the last session of 15 trials. Shown are means+SEMs. * $P \leq 0.05$ between groups.

rats to discriminate large from small rewards per se or their sensitivity to rewards. A limitation of the present study is that these possible underlying confounds were not controlled for. However, ample data exist showing that anxiety does not interfere with the ability to discriminate large from small rewards per se or the sensitivity to rewards. For example, Roman low-avoidance rats, that is high anxiety rats, did not differ in learning to discriminate large from small rewards in a delay-discounting task in the absence of a delay compared to Roman high-avoidance rats, that is low anxiety rats (Moreno et al., 2010). Unpublished data from our laboratory show that rats learned to discriminate high from low rewards (T-maze (5 vs. 1 sugar pellets) and r-IGT-like setup (3 vs. 1 pellets)) regardless of anxiety level as determined with the EPM. Finally, in rats rates of cocaine intake were not affected by anxiety levels as determined with the EPM (Bush and Vaccarino, 2007). The decreased win-stay behaviour in high anxiety subjects in the r-IGT may thus imply an altered perception of rewards in the context of the task, that is, in the context of uncertainty about the probability of wins and losses associated with the options. High anxiety subjects may have a shifted bias towards responding to the negatively valued stimuli, that is the quinine pellets in the r-IGT, and an underappraisal of the positively valued stimuli, that is the sucrose rewards leading to suboptimal decision-making.

An alternative explanation for the impaired decision-making in high anxiety rats needs to be considered as well. High anxiety subjects may perform worse on the r-IGT as a result of working memory deficits and therefore have problems incorporating previous outcomes in their subsequent choices. Humans with impaired working memory were found to perform worse on the IGT (Bechara et al., 1998; Suhr and Hammers, 2010). The literature on the effects of trait anxiety on working memory is inconsistent (Eysenk, 1979; Sorg and Whitney, 1992). High anxiety subjects have been found to outperform low anxiety subjects on a working memory task under nonstressful conditions (Sorg and Whitney, 1992), while we reported no relationship between trait anxiety and the Wisconsin Card Sorting Task (de Visser et al., 2010a). Rats selectively bred for high anxiety were not different from their low anxiety counterparts in a modified version of the hole board that tested for working memory (Ohl et al., 2002). Although a more indirect finding, neural cell adhesion molecule (NCAM)-deficient mice that showed impaired working memory showed diminished anxiety-like behaviour on the EPM (Jurgenson et al., 2010). Thus, both the human and animal studies do not show a clear relationship between levels of anxiety and working memory and accordingly it seems unlikely that working memory deficits are the main factor driving the impairments in decision-making in high anxiety subjects. However, future studies should be directed at finding a more conclusive answer.

Role of key brain areas in anxiety and decision-making

The present study provides for the first time insights in the neural regulation in IGT-like decision-making in rats in relation to behavioural traits, in this case anxiety. In male rats, decision-making performance was directly related to neural activity in the mPFC and striatum. More specifically, "poor" performers showed an increased level of c-fos expression in the pre- and infralimbic cortices and the nucleus accumbens shell compared to "good" performers. Interestingly, these brain areas were also found to regulate IGT-performance in humans and more specifically they are

thought to play a role during the initial exploratory phase of the task (Bechara et al., 1999; Ernst et al., 2002; Bolla et al., 2004; Ridderinkhof et al., 2004; Brand et al., 2006; van den Bos et al., 2006a, 2007; Doya, 2008; Lawrence et al., 2009; Li et al., 2010). Notably, "poor" performers still showed an overall preference for the advantageous option, only less strongly so than "good" performers. This leads to the suggestion that in male rats "poor" performers show suboptimal rather than impaired decision-making and may still be in the more exploratory phase of the task where immediate choice outcomes and the formation of behavioural programmes dominate the observed choice pattern. By contrast, increased levels of neural activity in the nucleus accumbens core were found in "good" performers compared to "poor" performers, suggesting distinct roles for the nucleus accumbens subareas in rat decision-making. As Yin et al. (2008) suggested, the nucleus accumbens core may be involved in more advanced decision-making processes than the shell, thus reflecting the more advanced performance of the "good" decision-makers. Moreover, the nucleus accumbens core has been implicated in impulse control and behavioural flexibility (Pothuizen et al., 2005; Floresco et al., 2006, but see Murphy et al., 2008), which may suggest that "good" decision makers are better at developing a behavioural strategy that is directed to the long-term gain of the advantageous option as opposed to the immediate gain of the disadvantageous option.

Correlation analysis further complemented these findings by revealing a negative correlation in male rats between final r-IGT performance, open arm exploration and activity in the nucleus accumbens shell. This implies a crucial role for the nucleus accumbens in regulating both decision-making and anxiety. This finding is supported by studies in rats showing an anxiolytic effect of GABA-agonists and AMPA receptor antagonist microinjections in specifically the nucleus accumbens shell (Lopes et al., 2007; Da Cunha et al., 2008). In these studies, lowered activation of the shell resulted in diminished anxiety-related behaviours on the EPM indicating the shell's contribution to the processing of aversive events.

No differences in neuronal activity were found in other areas of the prefrontal cortex, that is the Cg1, insular or OFC. These findings echo those of a recent study in which the mPFC was shown to be involved in proper performance in a probabilistic discounting task, which shares characteristics with the r-IGT (St Onge and Floresco, 2010). In that study, inactivation of the OFC, Cg1 and insular had no major effects on task performance. Indeed, the OFC seems to be more involved in delay-discounting (Mobini et al., 2002; Winstanley et al., 2004; Rudebeck et al., 2006) while the Cg1 is more involved in effort-related decision tasks (Walton et al., 2003; Rudebeck et al., 2006; Floresco and Ghods-Sharifi, 2007). In this respect, it would be relevant to test whether the OFC is involved in recently developed r-IGT paradigms that use delays as punishment (Rivalan et al., 2009; Zeeb et al., 2009).

Overall, our findings therefore suggest that high anxiety is accompanied by an altered frontostriatal circuitry, leading subjects to respond inappropriately when they are confronted with uncertainty on the probability and magnitude of wins and losses, which impairs their ability to make optimal decisions. Current experiments are directed at delineating this in more detail by using targeted inactivations of mPFC and ventral striatum in high and low anxiety subjects to measure the effects on r-IGT performance. Preliminary results indeed suggest a crucial role for the mPFC in affecting both anxiety behaviour on the EPM and performance in the r-IGT (de Visser et al., 2010b).

CONCLUSION

Anxiety affects decision-making processes in the r-IGT in rats in a similar way as in humans: subjects with higher anxiety profiles perform worse on the IGT/r-IGT. The medial prefrontal cortex as well as the ventral striatum may play a pivotal role in this interaction.

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