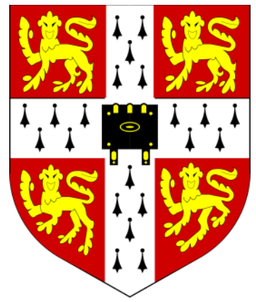
Effects of limbic corticostriatal lesions on autoshaping performance in rats

BJ Everitt, JA Parkinson†, G Lachenal, KM Halkerston, N Rudarakanchana, RN Cardinal, J Hall, CH Morrison, JW Dalley, SR Howes, TW Robbins
Departments of Experimental Psychology and †Anatomy, University of Cambridge, UK



Abstract. It has previously been shown that acquisition of Pavlovian conditioned approach behaviour (autoshaping) in rats depends on a circuit involving the anterior cingulate cortex (Ant Cing), the nucleus accumbens core (AcbC), accumbens dopamine (DA) innervation, and the central nucleus of the amygdala (CeN). This study sought to determine which elements of this circuit are required for performance of the task in well-trained animals. Rats were first trained that one visual stimulus, the CS+, was always followed by food, while another, the CS–, never predicted food. As a consequence, they came to approach the CS+ selectively. Subjects that reached a performance criterion subsequently received excitotoxic lesions of the Ant Cing (lesion $n = 11$ / sham 6), AcbC (ns = 9 / 7), or CeN (8 / 7), or 6-OHDA-induced dopamine depletion of the entire nucleus accumbens (7 / 7). Subjects were then re-tested.

Ant Cing lesions and AcbC lesions both impaired autoshaping performance. Acb DA depletion also impaired performance and the impairment was correlated with the degree of dopamine depletion, though the deficit was mild compared to that observed for the acquisition of autoshaping (Everitt *et al*., 1999). In contrast, rats with CeN lesions were unimpaired. These results support the view that the limbic corticostriatal circuit involving Ant Cing and AcbC is involved in the storage and/or expression of appetitive stimulus–reward associations, but suggest a critical role for CeN in early learning, which may reflect its role as a controller of attentional resources (Holland & Gallagher, 1999).

Introduction

- Autoshaping is a phenomenon in which subjects approach a conditioned stimulus (CS) that predicts an appetitive outcome (such as food), even though this approach behaviour has no effect on food delivery (Brown & Jenkins, 1968).

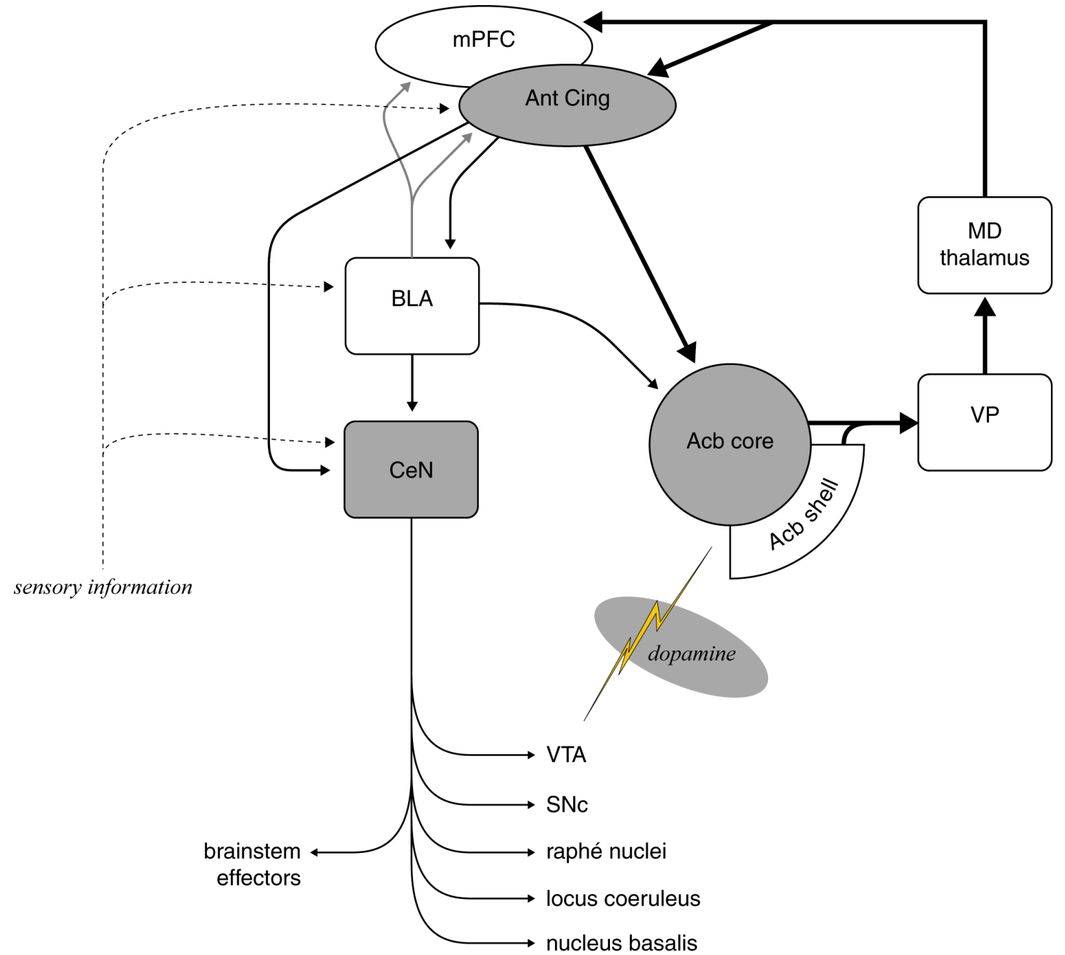
- Autoshaping reflects Pavlovian conditioned approach (Williams & Williams, 1969), and has been suggested to correlate with vulnerability to drug addiction (Tomie, 1996).

- In the autoshaping task used in our laboratory, two stimuli are used. A CS+ is presented, and followed by food delivery at a different spatial location. A second stimulus (CS–) is also presented, but never followed by food. Normal rats develop a discriminated approach response in which they approach the CS+ before returning to the food hopper to collect the reward.

- It has previously been shown that the *acquisition* of autoshaping depends upon the **Ant Cing** (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000b), the **AcbC** (Parkinson *et al.*, 2000b), the **CeN** (Parkinson *et al.*, 2000a), and the **dopaminergic innervation of the nucleus accumbens** (see Everitt *et al.*, 1999).

- However, it is not known whether these structures are simply required to learn the task, or whether they contribute to performance in a well-trained subject.

- The present study sought to address this issue. Rats were trained to a criterion on this task, received lesions of one of these structures, and were re-tested.



A simplified schematic of part of the 'limbic loop' of the basal ganglia. Grey shading indicates the regions lesioned in the present study. (*Abbreviations: Acb – nucleus accumbens; Ant Cing – anterior cingulate cortex; mPFC – medial prefrontal cortex; BLA – basolateral amygdala; CeN – central nucleus of the amygdala; VTA – ventral tegmental area; VP – ventral pallidum.*)

Methods

Autoshaping task

- Autoshaping was assessed in a testing chamber with a computer monitor on one wall and a centrally-located pellet dispenser.
- Subjects were trained to associate stimuli with the delivery of 45-mg sucrose pellets. The stimuli were 8 × 18 cm white vertical rectangles displayed on the left and right of the screen for 10 s. One was designated the CS+ and the other the CS–, counterbalanced across subjects.
- When the rat was located centrally at the rear of the chamber, a stimulus was presented. The CS+ was always followed by delivery of one food pellet; the CS– was never followed by food.
- Activation of a pressure-sensitive floor panel in front of a stimulus was scored as approach.
- A trial consisted of a presentation of the CS+ and one of the CS–, separated by a variable interval of at least 10 s. Rats were trained with 50 trials per day.

Experimental design

- Male hooded Lister rats were maintained at 80–90% of their free-feeding body weight.
- They were trained for 100 trials.
- Subjects that failed to approach the CS+ on ≥70% of the last 30 trials were given a further 50 remedial trials. Subjects that failed to meet this criterion on the last 30 remedial trials were excluded. (For the AcbC study, the criterion was approach to the CS+ twice as often as the CS– on the last 30 trials of training, with 30 or 60 remedial trials.)
- Successful subjects were assigned to groups for surgery.
- Following surgery, they were retested for a further 50 trials.

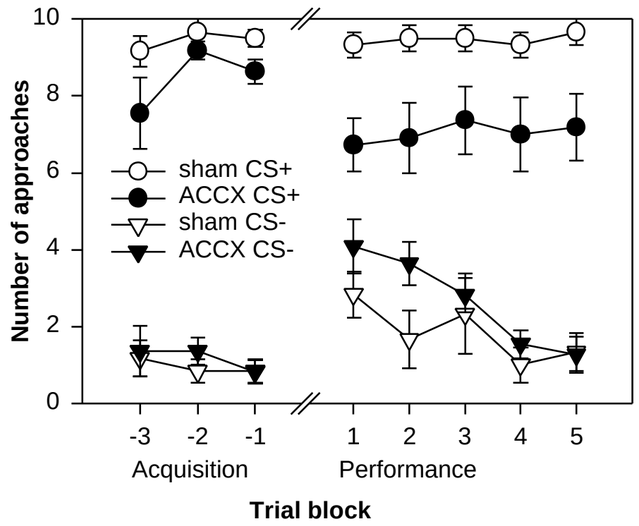
Excitotoxic lesions

- Excitotoxic lesions were made with 0.09 M quinolinic acid or 0.06 M ibotenic acid; sham-operated control rats were injected with vehicle only. Coordinates were taken from Paxinos & Watson (1998) with the incisor bar at –3.3 mm.
- For Ant Cing lesions, 0.5 µl quinolinic acid was injected at the following coordinates relative to bregma: AP +1.2, ML ±0.5, DV –3.0 and –2.2; AP +0.5, ML ±0.5, DV –2.8 and –2.0; AP –0.2, ML ±0.5, DV –2.5 and –2.0.
- For AcbC lesions, 0.5 µl quinolinic acid was injected at the following coordinates: AP +1.2, ML ±1.8, DV –7.1.
- For CeN lesions, 0.2 µl ibotenic acid was injected at the following coordinates: AP –2.2, ML ±4.0, DV –7.8 from dura; AP –2.7, ML ±4.0, DV –7.8 from dura.

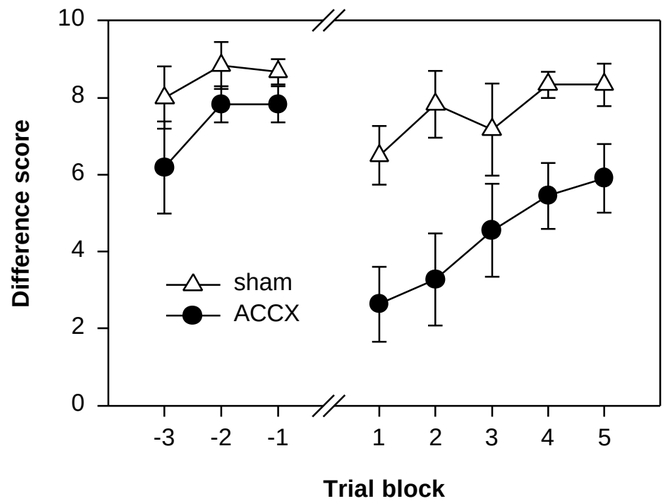
Neurochemical lesions and assessment

- Coordinates were taken from the atlas of Pellegrino *et al.* (1979), with the incisor bar set at +5.00 mm.
- Dopamine-depleting lesions of the nucleus accumbens (Acb) were made by injecting 2 µl of 23 µM 6-hydroxydopamine (6-OHDA) in 0.01% ascorbic acid at the following coordinates relative to bregma: AP +3.4, ML ±1.7, DV –7.2 from dura.
- After testing, subjects were killed by CO_2 inhalation and the brains were processed for neurochemical analysis. Tissue levels of noradrenaline (NA), dopamine (DA), serotonin (5-HT), the catecholamines metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined by reverse phase high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Samples of tissue were taken from the left and right nucleus accumbens (ventral striatum: VS) and from control areas, namely prefrontal cortex (PFC) and dorsal striatum (DS).
- Subjects that did not meet a criterion of ≥65% depletion of dopamine from the VS (that is, DA ≤ 30% of the mean level of the sham group) were excluded from the study.

1. Effects of lesions of the anterior cingulate cortex (Ant Cing)



Raw approach scores, pre- and post-operatively. (Breaks in the axis indicate the time of surgery.) Anterior cingulate lesions caused a persistent deficit in CS+ approach.



Difference scores (CS+ approaches minus CS– approaches) calculated for the same data. The ACCX group is significantly impaired.

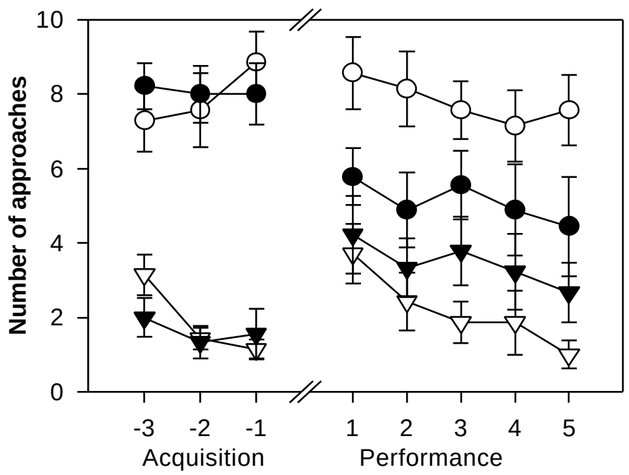
Ant Cing lesions impaired the performance of autoshaping.

Twenty subjects reached the initial performance criterion. Following histological analysis, the final group sizes were 11 (ACCX) and 6 (sham).

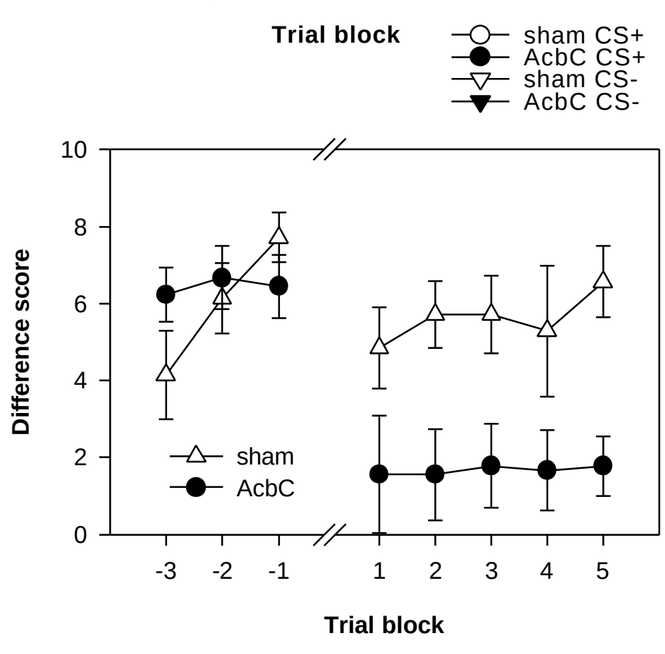
Pre-operatively, the groups did not differ.

Post-operatively, the ACCX group was significantly impaired relative to the sham group. Both groups showed a post-operative decline in CS– responding, and performance of the ACCX group did improve over testing. However, the ACCX group showed a persistent deficit in CS+ approaches.

2. Effects of lesions of the nucleus accumbens core (AcbC)



Raw approach scores, pre- and post-operatively. AcbC lesions caused a persistent deficit, particularly in CS+ approach.



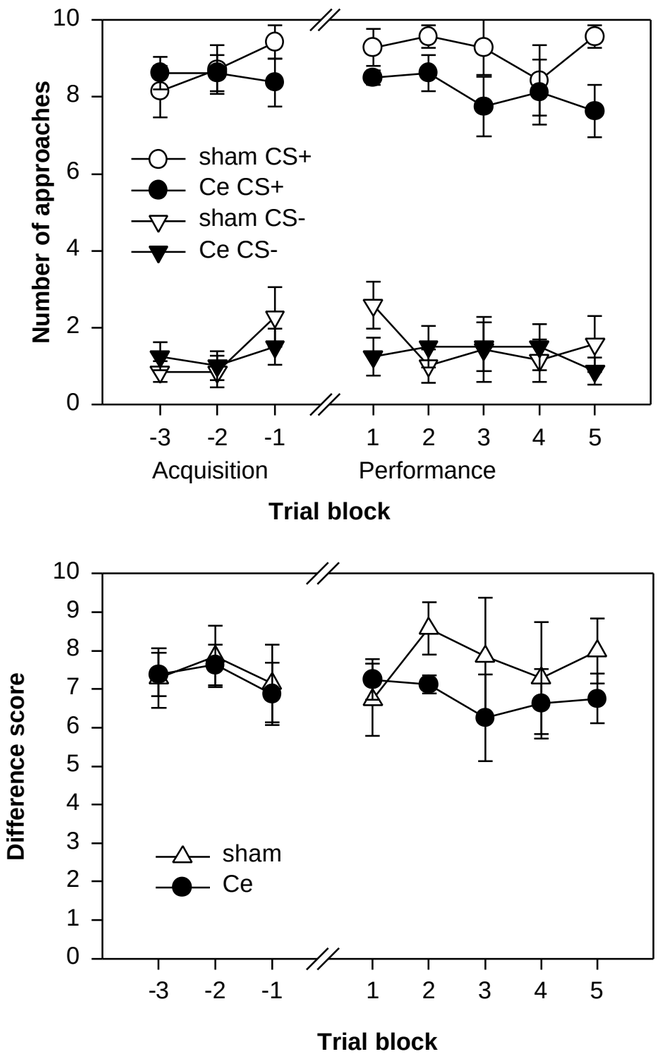
Difference scores calculated for the same data. The AcbC group is significantly impaired.

AcbC lesions impaired the performance of autoshaping.

Final group sizes were 9 (AcbC) and 7 (sham).

Though the groups did not differ pre-operatively, there was a severe and persistent impairment of performance in the AcbC-lesioned group. This group approached the CS+ significantly less than shams.

3. Effects of lesions of the central nucleus of the amygdala (CeN)



Raw approach scores, pre- and post-operatively. There was no deficit in CeN-lesioned rats.

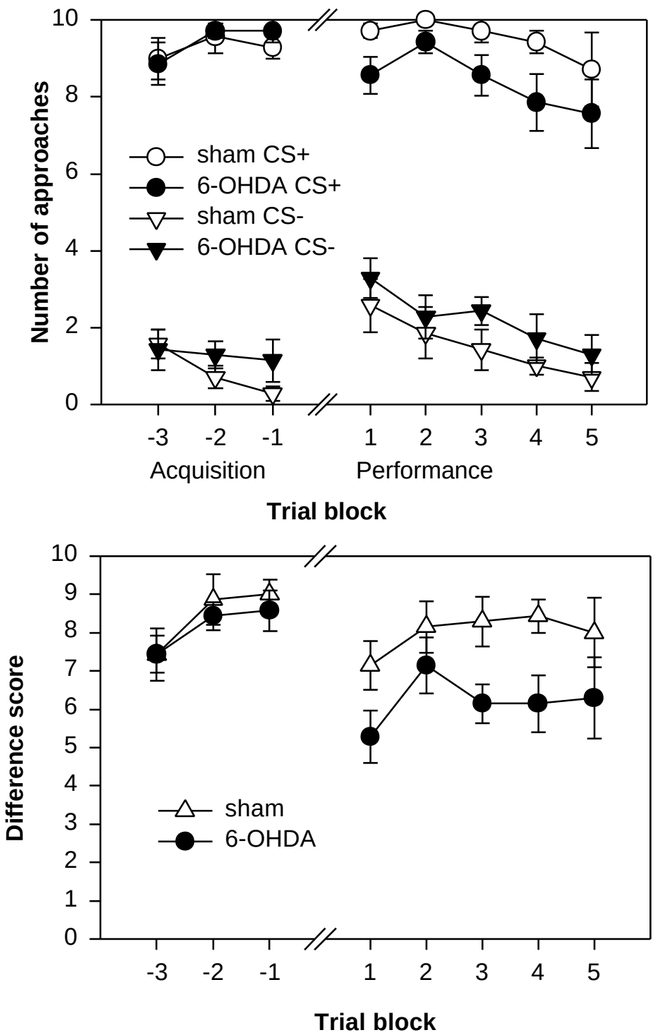
Difference scores calculated for the same data. There was no difference between the groups.

CeN lesions did *not* impair the performance of autoshaping.

Final group sizes were 8 (CeN) and 10 (sham).

The groups did not differ pre-operatively, and the lesion caused no deficit in performance.

4. Effects of dopamine depletion of the nucleus accumbens



Raw approach scores, pre- and post-operatively. Dopamine depletion of the nucleus accumbens induced a small but persistent reduction in CS+ approach.

Difference scores calculated for the same data. The 6-OHDA group is significantly impaired.

Dopamine depletion of Acb impaired the performance of autoshaping.

The groups did not differ pre-operatively. However, there was a small but persistent deficit in the 6-OHDA group post-operatively; the lesioned group approached the CS+ less often than shams.

- Final group sizes were 7 (6-OHDA) and 7 (sham).
- The lesion induced an 84% depletion of DA from the ventral striatum, with corresponding decreases in the metabolites DOPAC and HVA. Changes in NA, 5-HT and 5-HIAA were not significant.
- The lesion also depleted DA and DOPAC from the dorsal striatum, and NA and DOPAC from the PFC. No other differences were significant.
- There was a significant correlation between VS dopamine and autoshaping performance ($r^2 = 0.651$, $p = 0.009$).
- There was also a correlation of PFC NA with autoshaping performance, but when the correlation between PFC NA and VS DA was partialled out, the contribution of PFC NA was not significant ($p = .529$). No other correlations were significant.

Discussion and conclusions

Anterior cingulate cortex and nucleus accumbens core

- Lesions of the Ant Cing and AcbC both impaired the performance of autoshaping. Both these structures are also necessary for the acquisition of autoshaping (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000b), which requires a functional connection between the two (Parkinson *et al.*, 2000b).

- However, these structures probably make different contributions to conditioned approach behaviour. While the AcbC is required for many forms of conditioned approach (Parkinson *et al.*, 1999; Parkinson *et al.*, 2000b), the Ant Cing appears critical only when multiple CSs must be discriminated (see Cardinal *et al.*, this meeting, programme #366.13).

- Some recovery of function was observed in Ant Cing-lesioned rats, raising the possibility that the Ant Cing has a time-limited role in autoshaping, as has been suggested by other rodent studies (Freeman *et al.*, 1996; Gabriel, 1993; Gabriel *et al.*, 1980, p162; Hart *et al.*, 1997).

Dopamine innervation of the nucleus accumbens

- 6-OHDA-induced dopamine depletion of Acb impaired autoshaping performance by reducing the number of approaches to the CS+. This difference was not attributable to generally low levels of activity, as these animals were significantly hyperactive (data not shown).

- These data suggest that Acb DA contributes to the performance of a well-learned conditioned approach response. However, the impairment was much smaller than that observed when DA depletion occurs before training (see Everitt *et al.*, 1999), suggesting an additional role for Acb DA in learning.

Central nucleus of the amygdala

- Lesions of the CeN did *not* impair autoshaping performance.

- This result is in dramatic contrast to that of Parkinson *et al.* (2000a), who found that CeN lesions profoundly impaired the acquisition of the autoshaped response.

- These results suggest that the CeN, unique among the structures studied here, contributes selectively to *learning* of the conditioned approach response.

- One possibility is that the CeN is crucial for learning because of its role as an attentional controller. Several theories of learning postulate a role for attention in the formation of Pavlovian associations (Pearce & Hall, 1980; Sutherland & Mackintosh, 1971), while the CeN has been shown to be involved in the attentional regulation of stimulus associability (Gallagher & Holland, 1994; Holland & Gallagher, 1999), and to contribute to performance in other tests of attentional function (Holland *et al.*, 2000).

Acknowledgements

- Supported by the Medical Research Council (UK) and a Wellcome Trust Programme Grant.
- Conducted within the MRC Co-operative for Brain, Behaviour and Neuropsychiatry.
- RNC was supported by a research studentship from the UK Medical Research Council and a James Baird award from the University of Cambridge School of Clinical Medicine. JH was supported by Trinity College, Cambridge.
- Presented at the Society for Neuroscience 30th Annual Meeting, 4–9 November 2000, New Orleans, Louisiana, USA. (*Monday 6 November, 4–5 pm; programme #366.12; location MM-23.*)
- A PDF version of this poster will shortly be available at <**http://www.pobox.com/users/rudolf/publications**>.

References

Brown, P. L. & Jenkins, H. M. (1968). Auto-shaping of the pigeon's keypeck. *Journal of the Experimental Analysis of Behavior* **11**, 1-8.

Bussey, T. J., Everitt, B. J. & Robbins, T. W. (1997a). Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian autoshaping procedure for the rat: implications for the neurobiology of emotion. *Behav Neurosci* **111**(5), 908-19.

Everitt, B. J., Parkinson, J. A., Olmstead, M. C., Arroyo, M., Robledo, P. & Robbins, T. W. (1999). Associative processes in addiction and reward - The role of amygdala-ventral striatal subsystems. *Annals of the New York Academy of Sciences* **877**, 412-438.

Freeman, J. H., Jr., Cuppernell, C., Flannery, K. & Gabriel, M. (1996). Limbic thalamic, cingulate cortical and hippocampal neuronal correlates of discriminative approach learning in rabbits. *Behav Brain Res* **80**(1-2), 123-36.

Gabriel, M. (1993). Discriminative avoidance learning: a model system. In *Neurobiology of cingulate cortex and limbic thalamus: a comprehensive handbook* (Vogt, B. A. & Gabriel, M., eds.), pp. 478-523. Birkhauser, Boston, Massachusetts.

Gabriel, M., Foster, K., Orona, E., Saltwick, S. & Stanton, M., Eds. (1980). Neuronal activity of cingulate cortex, anteroventral thalamus, and hippocampal formation in discriminative conditioning: encoding and extraction of the significance of conditional stimuli. Vol. 9. Progress in Psychobiology and Physiological Psychology. Edited by Sprague, J. M. & Epstein, A. N.: Academic Press.

Gallagher, M. & Holland, P. C. (1994). The amygdala complex: multiple roles in associative learning and attention. *Proc Natl Acad Sci U S A* **91**(25), 11771-6.

Hart, M., Poremba, A. & Gabriel, M. (1997). The nomadic engram: overtraining eliminates the impairment of discriminative avoidance behavior produced by limbic thalamic lesions. *Behav Brain Res* **82**(2), 169-77.

Holland, P. C. & Gallagher, M. (1999). Amygdala circuitry in attentional and representational processes. *Trends in Cognitive Sciences* **3**, 65-73.

Holland, P. C., Han, J. S. & Gallagher, M. (2000). Lesions of the Amygdala Central Nucleus Alter Performance on a Selective Attention Task. *Journal of Neuroscience* **20**, 6701-6706.

Parkinson, J. A., Olmstead, M. C., Burns, L. H., Robbins, T. W. & Everitt, B. J. (1999). Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by d-amphetamine. *J Neurosci* **19**(6), 2401-2411.

Parkinson, J. A., Robbins, T. W. & Everitt, B. J. (2000a). Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. *European Journal of Neuroscience* **12**(1), 405-413.

Parkinson, J. A., Willoughby, P. J., Robbins, T. W. & Everitt, B. J. (2000b). Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: Further evidence for limbic cortical-ventral striatopallidal systems. *Behavioral Neuroscience* **114**(1), 42-63.

Paxinos, G. & Watson, C. (1998). *The Rat Brain in Stereotaxic Coordinates*. Fourth edit, Academic Press.

Pearce, J. M. & Hall, G. (1980). A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychol. Rev.* **106**, 532-552.

Pellegrino, L. J., Pellegrino, A. S. & Cushman, A. J. (1979). *A Stereotaxic Atlas of the Rat Brain*, Plenum, New York.

Sutherland, N. S. & Mackintosh, N. J. (1971). *Mechanisms of Animal Discrimination Learning*, Academic Press, New York.

Tomie, A. (1996). Locating reward cue at response manipulandum (CAM) induces symptoms of drug abuse. *Neurosci Biobehav Rev* **20**(3), 505-35.

Williams, D. R. & Williams, H. (1969). Auto-maintenance in the pigeon: sustained pecking despite contingent nonreinforcement. *J. Exp. Anal. Behav.* **12**, 511-520.