The role of the Amygdala in the perception of reward

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Declaration.

The work contained in this thesis was carried out by the author in the academic year 1993-1994 whilst a postgradute student in the Department of Psychology at the University of Durham. None of the work contained in this thesis has been submitted in candidature for any other degree.

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CONTENTS:

ABSTRACT:

This study set out to examine the role of the amygdala in a number of appetitively motivated tasks. Experiment one was a position discrimination task with reversals, which in later reversals involved manipulation of some secondary reinforcers associated with a correct response, and the introduction of a magnitude of reward component. Rats with NMDA-induced amygdala lesions performed at a similar level to shams at the initial discrimination and first three reversals, proceeding to reverse faster than controls in the subsequent three reversals. Manipulation of secondary reinforcers led to an equal and significant decline in performance for both groups, with the lesioned animals retaining their significant superiority in reversal performance. Alteration of the task from a 2 vs 0 pellet discrimination to a 2 vs 1 led to a drastic increase in task difficulty, but both groups completed three reversals and did not differ significantly in performance. Experience of handling the lesioned animals led to the confirmation, in experiment two, that they were significantly more hostile/reactive to handling than shams (using the "blind" ratings of experienced animal handlers). Experiment three attempted to refine the picture of this behavioural change by measuring gross activity levels - no differences between groups were found. The finding of enhanced reversal performance and the absence of a magnitude of reward deficit amongst lesioned animals in experiment one were unanticipated, problematic and demand replication. No strong support was provided for either of the principal contemporary theories of amygdala involvement in secondary reinforcement. Increased reactivity to handling was found to be consistent with a minority of the past literature, and activity levels were as anticipated. It is argued that the notion of "stimulus-reward associations" as an amygdala function is incoherent and unhelpful, and that references to the functions of the amygdala as a whole rather than of subnuclei can be equally misleading.

1.1 - An introduction to the neuroanatomy of the rat amygdala

The amygdala is named after the almond, which (in the minds of early anatomists) it resembles. In humans it can be found lying medially in the anterior portion of the temporal lobes, anterior to the hippocampus. Although it was not included in Papez's (1934) original proposal for a "limbic system", it is now considered an important part of this hypothesised circuit, and its perceived functional and clinical significance have increased drastically in the last ten to fifteen years.

The amygdala's location in most mammals is characterised as anterior to the temporal tip of the horn of the lateral ventricles (Isaacson, 1974). In the rat, it is situated ventrally and laterally, between the inferomedial aspect of the cortex and the lateral border of the hypothalamus. Its rostro-caudal extent is approximately the same as the hypothalamus, though in fact the hypothalamus is somewhat longer (rostrally).

The amygdala is not a unitary structure. Traditionally, (Johnston, 1923) the amygdala has been divided into two main groups of nuclei, the corticomedial and the basolateral. Modern neuroanatomy has been able to distigiush over ten subdivisions in fact, but the earlier dichotomy remains useful when considering lesion research, as most individual nuclei are too small to lesion on their own. The only modification to this scheme might be to separate the central nucleus into a third category, as it is quite distinctive in its connective features (e.g. heavy hypothalamus/brainstem projections); this tripartite sketch will prove to be a useful heuristic when considering research.

Until roughly thirty years ago, the pricipal connections of the amygdala were thought to be with the hypothalamus. This view has changed radically, with the demonstration of profuse and often reciprocal connections with the cortex, thalamus and striatum, along with the basal forebrain, brainstem and hippocampus. The discovery and exploration of striatal connections has proved particularly important in the investigation of processes related to reward - see section 3.33. The complexity of the picture is increased by the intricate array of intrinsic connections that link individual nuclei to one another. Neurochemically, the amygdala is characteristic and fascinating; a remarkable array of peptides, monamines and amino acids are present in it.

Having completed this thumbnail sketch of amygdala anatomy, I plan to list the individual nuclei, and describe their general cytoarchitecture, the cells types present and their neurochemistry.

Finally, I will cover the neurocircuitry of the amygdala - its intrinsic connections, and its afferents and efferents, both cortical and subcortical.

1.2 - THE AMYGDALOID NUCLEI.

For this section, I will list the nuclei so that the terms are familiar to the reader throughout the rest of this section. It is important at this point to initiate a consistent terminology and set of abbreviations, as there have been many different taxonomies of amygdaloid nuclei over the years. Price et al.'s (1987) authoritative review is my source for this. I will add a descriptive or explanatory note and some description of cytoarchitecture where appropriate, but the interested reader is referred to Price et al.'s (1987) detailed summary for more detailed diagrams, or to appendix one.

The ultimate goal of much research into amygdala function is to produce results relevant to the scientific understanding and clinical manipulation of the human brain. The "limbic system" is evolutionarily older than the neocortex, and consequently it is regarded as relatively easy (or relatively reliable) to generalise results across species boundaries. Despite this, amygdaloid nuclei differ in relative size across species, and this must be borne in mind when hoping to draw any valid rat-human comparisons; consequently, this data is highly relevant. Stephan and Andy (1977) have studied the relative sizes of amygdaloid nuclei in animals ranging from insectivores to simians. The "centromedial" group (diverging slightly from the divisions suggested earlier) shrinks, and the basolateral group can be seen to swell as one "ascends" the primate scale in the direction of greater encephalisation. Comparing monkeys to rats, the lateral and basolateral nuclei are larger in the monkey, whereas the medial nucleus is notably bigger in the rat.

LIST OF NUCLEI:

(a) Nucleus of the lateral olfactory tract - **NLOT**

This nucleus is a significant feature of the rostral pole of the amygdala. Cytoarchitecturally, it consists of three distinguishable layers, and functionally it is part of the huge olfactory input into the amygdala which is so prominent in rats.

(b) Anterior cortical nucleus - **COa**

(c) Medial nucleus - **M**

This nucleus is prominent and well defined in the rat. A rostral subdivision (**Mr**) can be found (exclusively) in the rat which is quite cytoarchitecturally distinct, and is the main area of the medial

nucleus which projects to the ventromedial hypothalamus. The caudal subdivision is larger and denser (Mc).

(d) Bed nucleus of the accessory olfactory tract - BAOT

(e) Periamygdaloid cortex - PAC

This is a large area on the ventral surface of the amygdala, bordering on the pirifrom and entorhinal cortices, amygdalo-hippocampal area and anterior cortical nucleus. It is principally a molecular layer of cells; a subdivision is the sulcal region (adjacent to the incipient amygaloid sulcus), PACs.

(f) Posterior cortical nucleus - COp

(g) Basal Nucleus - B

This corresponds roughly to what has most commonly been described as the basolateral nucleus (i.e. Krettek & Price, 1978.) It nearly touches both the anterior and posterior boundaries of the amygdala, and has magnocellular (Bm) and parvicellular (Bpc) subdivisions.

(h) Accessory basal nucleus - AB

In the rat, this nucleus has previously been called the basomedial nucleus. It has a superficial division (ABs).

(i) Lateral nucleus - L

This nucleus is quite large in the rat, especially in the posterior area of the amygdala.

(j) Central nucleus - C

This is a large, functionally significant structure, with efferent fibres exiting the amygdala via the stria terminalis and the ventral amygdalofugal pathway. Four subdivisions can be observed in the rat, though Price et al. (1987) only separate two, in order to maintain consistent terminology with the cat and monkey. These are the medial (Cm) and the lateral (Cl) divisions, the former being the larger but less dense of the two. Many other amygdaloid nuclei project to this structure, as will be seen in a later section.

(k) Bed nucleus of the stria terminalis - BNST

The name of this structure is essentially self-explanatory. Descending fibres from the stria terminalis (which leaves the amygdala at its caudal pole) pass through this structure to the preoptic region and the hypothalamus, and the BNST is also the source of many of these strial fibres. Medial and lateral divisions are separated by commisural fibres. Johnston (1923) was the first to point out that the BNST

was actually a rostral section of the amygdala; it is in fact separate from the main body of amygdaloid nuclei, but he demonstrated that it develops from the same rudiment as C and M, using both phylogenetic and ontogenetic evidence.

(l) Amygdalo-hippocampal area - AHA

This is situated, unsurprisingly, between the posterior pole of the amygdala and the ventral subiculum of the hippocampus (HPC).

(m) Paralaminar nucleus - PL

(n) Intercalated nuclei - I

Also; Endopiriform nucleus (En), anterior amygdaloid area (AAA) which merges into the hypothalamus at its medial border, piriform cortex (PC).

1.3 - CELL TYPES PRESENT IN THE AMYGDALA

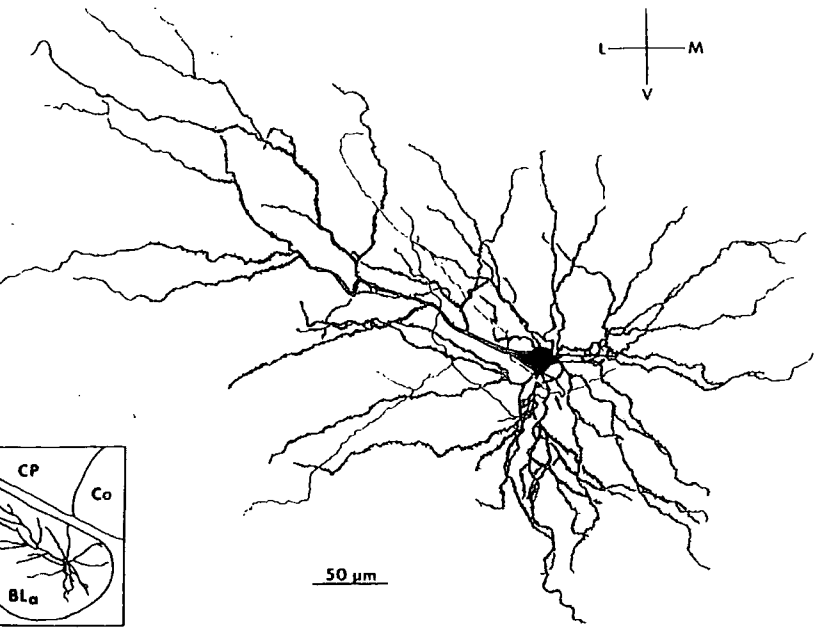
In terms of cell types, the lateral, basal and accessory basal nuclei can be grouped together and described generally. Approximately 70% of the cells found in these regions are of a type quite similar to cortical pyramidal cells, and have subsequently been named "pyramidal" by McDonald (1992). They are the same as those described as class I "spiny" cells by Mc Donald (1982, 1984) and closely resemble the "P cells" that Hall identified in cats in 1972. They typically have 3-5 dendrites, one of which is markedly thicker than the others and resembles an "apical" dendrite (see fig 1.1). Terminal ramification is absent in these cells (distinguishing them from cortical pyramidals) and they are oriented rostrocaudally, the apical dendrite pointed rostrally. Although classed here as a "type" of cell, there is in fact considerable morphological variation in different areas of the amygdala (i.e. some cells have two apical dendrites), and so any one cell of this type is best seen as one point on a continuum.

The other class of cells to be found in these nuclei (McDonald 1982/84 class II, "stellate"; Hall 1972 "type S") are described by McDonald (1992) as "nonpyramidal" cells. These have smaller, generally ovoid somata, and lack dendritic spines and the apical dendrite seen in pyramidal (class I) cells (fig 1.2). Again, this is a morphologically heterogeneous group; multipolar, bitufted and bipolar variants can be found.

The central nucleus has its own distinguishing cytoarchitecture, and consequently the cells of the medial and lateral divisions must be described separately. The cell bodies of medial division neurons are ovoid, fusiform or piriform in shape (fig 1.3). Their dendritic structure is comparatively

complex, with 3-4 non-spiny primary dendrites, and a number of rectilinear non-spiny secondary dendrites; cells of the medial nucleus are similar to these. The primary axon originating from these cells often projects ultimately to the stria terminalis or the ventral amygdalofugal pathway. Equally complex are lateral division cells, which are spiny-stellate, and often resemble some cells which can be found in the striatum, particularly the laterally adjacent putamen (Hall, 1972). Primary dendrites number three to five having few spines, and there are secondary and tertiary dendrites which typically become more spiny (*very* spiny, fig 1.4) distally.

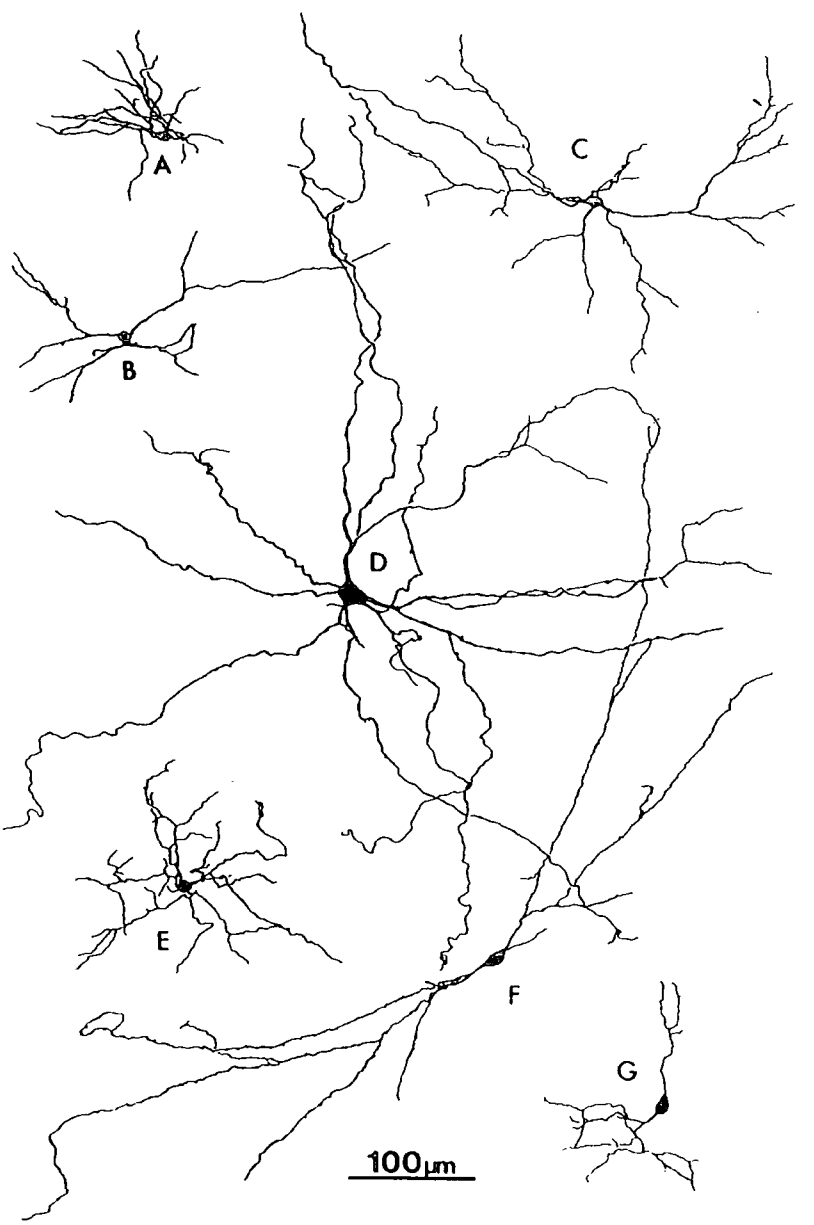
Hall (1972) regards the cortical and basal nuclei as anatomically continuous (although they are clearly functionally differentiated), and the cells of the cortical nucleus also closely resemble those of the adjacent piriform cortex. Most of these are spiny pyramidal cells, and as in the basal nucleus there are also scatterings of the "non-pyramidal" type. As mentioned above, the cells of the medial nucleus are similar to those found in the medial division of the central nucleus, and most studies have only found cells of this type to be present; the boundary between cortical and medial nuclei is therefore quite easy to spot. The neurons of the BNST resemble central nucleus cells, and also the NLOT consists mainly of the class I "pyramidal"/"spiny" cells found in the lateral and basal nuclei.



⇐ FIG 1.1

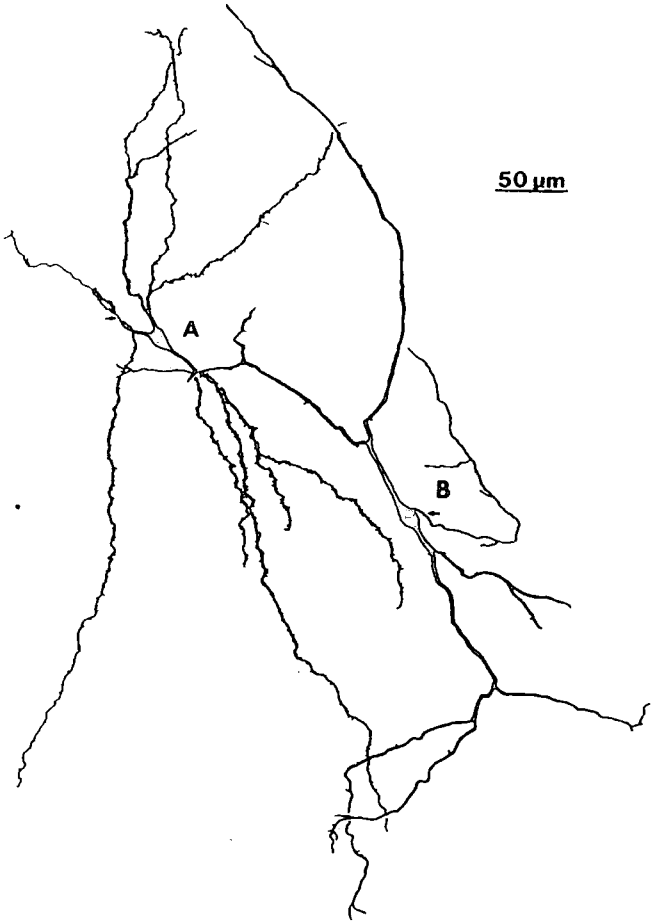
A Golgi-impregnated pyramidal neuron in the magnocellular basal nucleus of rat (anterior division of the basolateral nucleus of Krettek and Price, 1978b). Note midal shape of the cell and the presence of one apical dendrite and numerous basal rites. Arrow indicates axon in this and all subsequent figures. Inset shows position ll. Cross indicates orientation.

FIG 1.2 ⇒



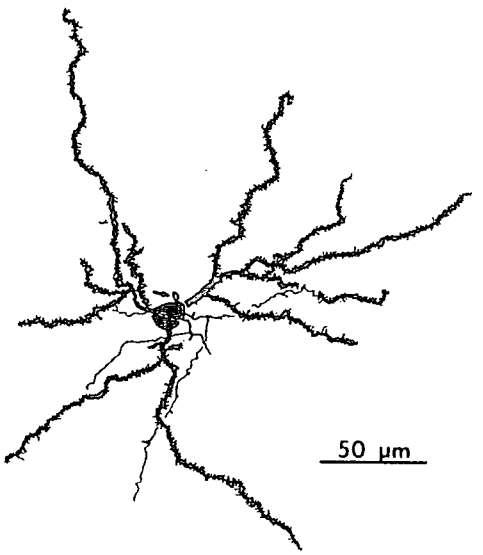
Drawings of Golgi-impregnated nonpyramidal cells that best illustrate the morphological variation of these neurons in the rat basolateral amygdala. Only cell bodies and dendrites of these neurons are illustrated.

FIG 1.3 ⇒



Golgi-impregnated neurons of the medial subdivision of the central nucleus in the rat. Dendrites of cell A are thin and have a moderate covering of spines, while cell B has thick dendrites with virtually no dendritic spines.

FIG 1.4 ⇒



Golgi-impregnated medium-sized spiny neuron of the lateral subdivision of the central nucleus in the rat.

1.4 - NEUROCHEMISTRY OF THE AMYGDALA

In my introduction, I alluded to the wide range of neuroactive substances present in the amygdala. The distribution of these substances could, at some point in the future, tell us as much about amygdala function as the amygdala's neural connections. I am going to adopt two different approaches for the localisation of these compounds; for monoamines and cholinergic chemicals, I will state the chemical and then its location, for the peptides, I will state the location and then list the peptides present

Cholinergic compounds (acetylcholine -ach-, ach'esterase, ach'transferase, for example) are to be found mainly in the lateral and basal nuclei (Ben-Ari et al., 1977), the source of inervation for cholinergic compounds being the substantia innominata. The monoamines are widely present in the amygdala, particulary dopamine (DA) and noradrenalin (NA). DA is found principally in the central and lateral subdivisions of the amygdala[1], sources for this innervation being the ventral tegmental area, substantia nigra and pars compacta (Fallon et al., 1978). Noradrenaline is focussed in the "basolateral group", the noradrenergic neurons appearing to originate in the locus coeruleus and the lateral tegmental group. Serotonin (5-HT) is "distributed loosely" amongst the various nuclei, coming in from the mesencephalic pontine raphé nuclei.

γ-amino-butyric acid (GABA), glutamic and aspartic acid are amino acid neurotransmitters. GABA is most concentrated in the central and medial nuclei (Ben-Ari et al., 1976, Ottersen et al. 1986) and is also present in the intraamygdaloid portion of the BNST, and the AAA. Corticoamygdaloid fibres appear to utilise glutamate and aspartate (Ottersen et al., 1986), and this may help explain their distribution in the lateral, basal, and anterior cortical nuclei, as well as the PAC. They are found in lower concentrations in the more medial amygdaloid nuclei. The neurotoxin N-methyl D-aspartate (NMDA, see section 5.1) acts at glutamate receptors to kill cell bodies.

It is easiest to describe the peptides in terms of their location in the amygdala. The lateral, basal and accessory basal nuclei have been found to contain cholecystokinin (CCK), vasoactive intestinal peptide (VIP), and somatostasin (SOM) (McDonald, 1985.) The cortical and medial nuclei, along with the PAC, can all betreated as a group in terms of peptide distribution. They contain CCK,

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[1]For the functional significance of the presence of dopamine, see Hori et al.'s (1993) experiment on discrimination learning.

VIP, SOM, neurotensin (NT), enkephalin (ENK), and the medial nuclues contains substance P (SubP).

The extraordinary abundance of central nucleus peptides has already been alluded to; indeed, it counts as one of the richest assortments of such chemicals in any single brain structure. The most significant peptides present include NT, SOM, ENK, SubP, CCK, and a remarkable array of others (see Price et al. 1987). Cells that project to the brainstem often stain for SOM, NT, corticotrophin releasing hormone (CRH) and some SubP. The BNST contains a similar cauldron of chemicals to the central and medial nuclei, with an intriguing structural correlation - substances found in the lateral nucleus of the BNST will similarly be found in the lateral parts of the central nucleus.

It is important to note the distribution of steroids in the amygdala particularly in the light of Meaney and McEwan's (1986) intriguing data on the consequences of testosterone infusion into the amygdalae of juvenile female rats. The testosterone "masculinised" the play behaviour of the treatment animals, implying a major role for steroid-concentrating cells in the amygdala in influencing behaviour. Cells that concentrate gonadal hormones can be found in the cortical and medial nuclei, and the PAC and AHA.

1.5 - AMYGDALOID AFFERENTS AND EFFERENTS:

The amygadala's connections have been studied extensively in the rat, cat and monkey. Its multifarious target and afferent sites include the striatum, thalamus, hypothalamus, brainstem, pons & medulla, hippocampus and cortex. I wish to enumerate the exact sites in more detail in this section, but it is helpful to start with a description of the amygdala's two main efferent pathways; the stria terminalis and the ventral amygdalofugal pathway. Awareness of the general pattern of efferent fibre pathways is helpful in comprehending the pattern of projection sites.

The course of the stria terminalis runs alongside the tail and body of the caudate nucleus. Two components can be distinguished, a dorsal and a ventral. The dorsal component of the stria terminalis arises principally from cell bodies in the medial and cortical nuclei. At roughly the level of the anterior commissure, the fibre bundle splits into four parts (see fig 2.1). The retrocommisural part terminates in the BNST and the preoptic region, and the commisural strand projects to the BNST ands to the *contralateral* cortical and medial nuclei. The fibres of the (third) hypothalamic component are in close association with those of the parolfactory division, until the latter terminates in the lateral

septal nucleus, the nucleus accumbens and the olfactory tubercule. Hypothalamic component fibres continue further to terminate in the medial preoptic region and the ventromedial hypothalamic nucleus.

Ventral component fibres show a more focussed pattern of termination. They issue from the medial and basal nuclei, and terminate in the BNST, preoptic nucleus and the core of the ventromedial hypothalamic nucleus (fig 2.2). Some reciprocal connections can also be found, arising from termination areas, and projecting to M and B.

The ventral amygdalofugal pathway is a fairly diffuse structure which runs along the ventral surface of the brain (fig 2.3). The PAC contains a large proportion of the cell bodies that give rise to this pathway, and it projects to a wide range of structures: preoptic nucleus, nucleus accumbens, diagonal band, anterior hypothalamus, and elsewhere via the medial forebrain bundle. There are also some reciprocal connections to the amygdala (amygdalo*petal* connections) arising from the preoptic nucleus and anterior hypothalamus. Amygdaloid projections also run along this general route to the mediodorsal nucleus of the thalamus (MD), but they arise from separate nulcei to the main pathway.

I intend to deal with afferents and efferents to cortical and subcortical structures separately. Graphic representations prove to be overcomplex and misleading, so I will list the names of the structures, and wherever possible name the amygdaloid nucleus of origin/termination.

Amygdaloid afferents, subcortical:

Thalamic sources.

Paraventricualar nucleus, nucleus reuniens, certain subdivisions of the nucleus centralis complex of Olszewski. TO: **B, C.**

Hypothalamic sources.

Widespread, especially the ventromedial nucleus, caudal levels of the lateral hypothalamic area, lateral preoptic area. TO: parvicellular divisions of **B, AB.**

Midbrain sources.

Ventral tegmental area, substantia nigra pas compacta, locus coeruleus, dorsal raphe nucleus, peripeduncular nucleus. TO: **C.** Dorsal raphé produces a serotonergic input that is spread quite diffusely among the nuclei.

Pons & Medulla.

Parabrachial nucleus.TO: **C.**

Hippocampus

Ventral subiculum, CA1, entorhinal cortex. TO: **C, Bpc, M.**

Substantia innominata. TO: NLOT, Bmg, AB

Olfactory bulb. TO: NLOT, COa, M, PAC, COp

Contralateral amygdala

Cortical:

Piriform, entorhinal (layers II & III) TO: NLOT, COa, PAC

Generally, the posterior part of the rhinal sulcus. Posterior insular cortex, perirhinal cortex.

Amygdaloid efferents, subcortical.

Thalamus:

Mediodorsal nucleus. FROM: all nuclei but C and M.

Nucleus reuniens, nucleus centralis complex of Olszewski. FROM: M, C.

Hypothalamus:

Anterior hypothalamus, preoptic region. FROM: BNST

Ventromedial nucleus. Core, FROM: M, AB. Shell, FROM: AHA.

Dorsal and ventral premamillary nuclei, supramamillary nuclei. FROM: as ventromedial, plus COp.

Striatum:

Nucleus accumbens, olfactory tubercule, dorsal neostriatum. FROM: B, AB

Midbrain:

Reticular formation, ventrolateral and dorsal periaqueductal grey. FROM: C.

Pons & Medulla:

Parabrachial nucleus, Mesencephalic nucleus of the trigeminal nerve, dorsal motor nucleus of the vagus nerve, nucleus of the solitary tract, "ventrolateral medulla." FROM: C.

Hippocampus:

Rostral entorhinal cortex, ventral subiculum, CA1. FROM: PAC, AB, (entorhinal) L.

Substantia innominata: FROM: Bpc, ABmg, C

Nucleus basalis of Meynert.

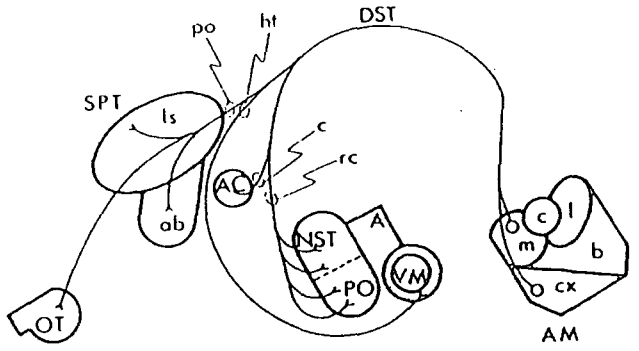
Contralateral amygdala.

Cortical:

Rhinal sulcus: Agranular and dysgranular insular cortex, perirhinal cortex. FROM: B, L, respectively.

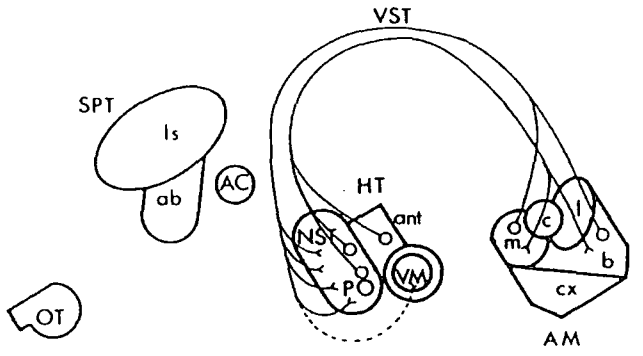
Medial and orbital prefrontal cortex, piriform cortex.

FIG 2.1 ⇒



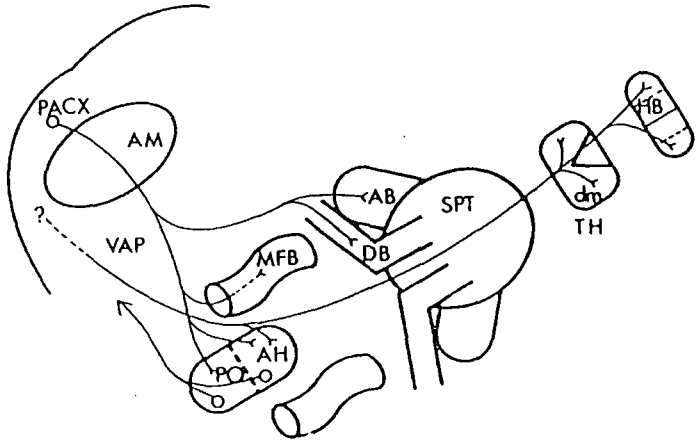
Summary of the projections of the dorsal component of the stria terminalis (DST). AM = amygdala (cx = cortical nucleus, m = medial nucleus, c = central nucleus, l = lateral nucleus, b = basal nucleus), po = parolfactory component (ls = lateral septum, ab = accumbens nucleus, OT = olfactory tubercle), ht = hypothalamic component (VM = ventromedial hypothalamus), c = commissural component (AC = anterior commissure), rc = retrocommissural component (NST = nucleus of the stria terminalis, PO = preoptic region).

FIG 2.2 ⇒



Summary of the projections of the ventral component of the stria terminalis (VST). NST = nucleus of the stria terminalis, PO = preoptic area, ant = anterior regions of the hypothalamus (HT), AM = amygdala (cx = cortical nucleus, m = medial nucleus, c = central nucleus, l = lateral nucleus, b = basal nucleus).

FIG 2.3 ⇒

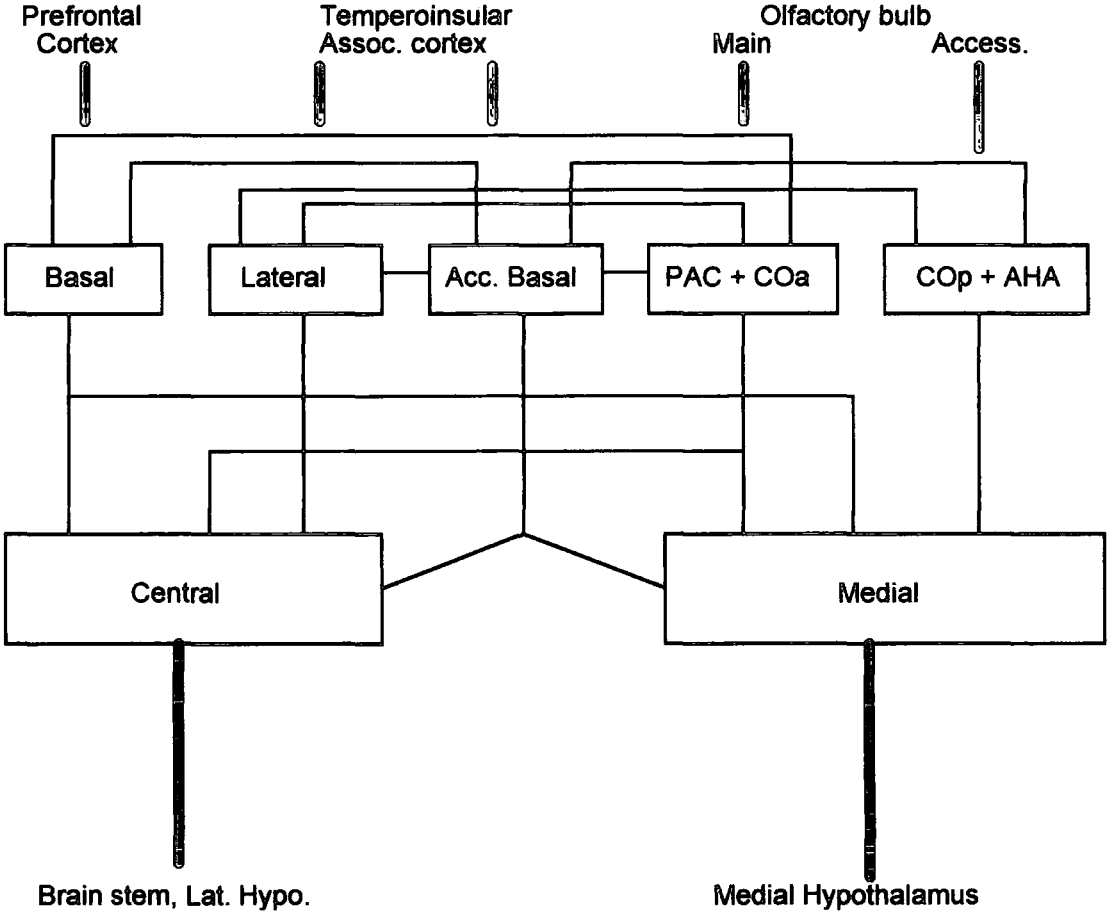


Summary of the projections of the ventral amygdalofugal pathway (VAP) or the amygdalopyriform association bundle. PACX = periamygdaloid cortex, AM = amygdala, PO = preoptic area, AH = anterior hypothalamus, MFB = medial forebrain bundle, DB = diagonal band, AB = accumbens nucleus, SPT = septal region, dm = dorsomedial nucleus of the thalamus (TH), HB = habenula.

1.6 - SCHEMATIC SUMMARY OF THE KNOWN INTRAAMYGDALOID CONNECTIONS:

When considering the intrinsic connections of the amygdala, it becomes evident that Johnston (1923) was not at all arbitrary in his division of the amygdala into basolateral and corticomedial sections. A more refined distinction which combines discrimination by cell type and by connectivity is cited by McDonald (1992), splitting the amygdala into cortexlike nuclei (B, L, AB, PAC, COa, COp, AHA) and noncortexlike nuclei (C, M, BNST). The grounds for a distinction like this are justified by study of cell types alone (see above), but the following diagram will make clear why it is also anatomically important.

FIG 3.1



This diagram is taken from McDonald (1992). The cortexlike nuclei are arranged from left to right, moving from more superficial to deeper structures. All of the cortexlike nuclei are closely interconnected, the one exception being that there are no connections between the lateral and basal

nuclei. Particularly heavy projections can be found from the lateral nucleus to the accessory basal nucleus and the periamygdaloid cortex.

The more superficial cortexlike nuclei connect principally to the central nucleus, the deepernuclie to the medial nucleus (as can be seen in fig 3.1). Central-medial connections do appear to exist, (at least in the monkey - Price et al. 1987), but have proved hard to verify due to these structures being so close to one another. Projections from the noncortexlike nuclei back to the cortexlike are negligible.

The nucleus of the lateral olfactory tract (NLOT) is not included in fig 3.1. This is classified as a cortexlike nucleus, and receives projections from the periamygdaloid cortex and the anterior cortical nucleus. These structures in turn receive the main olfactory bulb input to the amygdala. The basal nucleus also projects to the NLOT, and receives a reciprocal projection from it.

2.1 - SOCIAL, EMOTIONAL, REPRODUCTIVE AND OTHER SPONTANEOUS BEHAVIOURS.

This section is rather broad in conception, and I aim to offer a general overview of the input of the amygdala into "non-regulated", "natural" behaviours in the rat. Much of the seminal work on emotionality after temporal lobe damage was performed on monkeys, so I will start with a review of these experiments to provide historical context. The rest of the section will be loosely structured, covering emotional, social, reproductive and spontaneous behaviours in turn and recognising that there are no clear boundaries between these topics.

Before actually commencing, it is informative to quickly reconsider the amygdala's neural connections. It projects extensively to the hypothalamus and the brainstem, implying a possible role in governing autonomic and endocrine activity, and it receives projections from all of the unimodal sensory association areas, and some multimodal association areas (e.g. insula). This arrangement makes the notion of the amygdala as sensory "gateway" to the emotions very intuitively appealing; incoming sensory data would be processed in terms of the affective significance of the sense data, and emotional responses produced accordingly. One experiment in particular stands out as giving clear support to this idea. Downer (1961) performed a commisurotomy and unilateral amygdalectomy on a monkey. The result of this surgery was to ensure that visual stimuli presented to one eye could only be processed in the ipsilateral visual cortex and therefore only relayed to the ipsilateral amygdala. Stimuli were presented to the monkey which would normally induce fear; when presented to the eye which relayed information to the intact amygdala, the monkey showed a normal fear reponse, but due to the lesion of the other amygdala, fear was absent when the same stimuli were presented to the other eye. Clearly, the amygdala could be seen to be the site at which certain emotional responses are elicited from visual stimuli, and the gateway theory of amygdala function received some support.

The amygdala also projects back to the sensory cortex from which it receives so many afferents. Although the functional significance of this fact has not been empirically verified, it seems likely that there is feedback from the amygdala to sensory cortex, which allows the emotional state of the organism to influence perception.

In 1888, Brown and Schafer noted that monkeys that were normally fierce and aggressive became comparatively tame after temporal lobe lesions. This observation was confirmed and

elaborated by Kluver and Bucy, who, in 1937 carefully observed monkeys with widespread temporal damage and produced a reliable list of the consequences of these lesions which became known as the Kluver-Bucy syndrome. Weiskrantz (1956) refined this list to include just the sequelae of selective amygdala damage. Bilateral amygdalectomy in monkeys was found to produce the characteristic hypoemotionality, along with orality, dietary changes and hypermetamorphosis. These results have subsequently been replicated (Downer, 1961; Horel et al. 1975; Aggleton and Passingham, 1981). Such an impact on emotion cannot be observed after lesioning any other brain structure of comparable size, and consequently the amygdala is now regarded as critical in emotional processing and behaviour.[1]

This scanty historical survey omits a great deal, but the focus must be on rat studies for the rest of this section.

2.2 - AGGRESSION.

Aggressive behaviour undergoes significant changes after amygdalectomy; to permit a more detailed analysis, it must be noted that aggression is not a unitary phenomenon. I will consider such specifics as predatory[2], intermale and "defensive" aggression here.

In 1975&1976, Vergnes found that lesioning the medial nucleus or the stria terminalis produced an increment in predatory behaviour (mouse-killing). This can be contrasted with Hilton and Zbrozyna's 1963 study which showed that lateral amygdala provoked no change in predatory activity (also Vergnes 1976). More data is avaialble for intermale aggression and defensive attack; Bolhuis et al. (1984) found that rats that would normally be submissive to a dominant male (who had beaten them before) fought normally if they had suffered corticomedial damage. Luiten et al. (1985) confirmed that animals with corticomedial damage showed no fear response to dominanat males. Kemble et al. (1984) have sought to differentiate the functions of medial and cortical nuclei, finding that cortical nucleus damage led to less flight behaviour, and medial nucleus damage resulted in a reduction in defensiveness. The most elegant dissociation acheived so far in the study of this topic has been made by McGregor and Herbert (1992), who have shown that basolateral lesions significantly reduce aggressive inter-male behaviour, whereas corticomedial lesions do not. (The opposite was

[1]It should be noted that changes in emotion are not entirely consistent; both Aggleton & Passingham and Rosvold et al. report individual animals that show an *increase* in aggressive behaviour.
[2]it is questionable whether this should be classed as aggression at all.

found for male sexual behaviour - see section 2.4, below.) There is obviously some conflict with the findings of Luiten et al (1985) above with regard to the effects of corticomedial lesions.

Hilton and Zbrozyna (1963) have studied another category of aggression which they term "defensive/affective attack" and which occurs after a rat has received a footshock, for example. Lateral nucleus lesions reduced this type of attack, and stimulation of the basolateral group accentuated it, even if the stria terminalis was also cut. Female rats are protective towards their pups; if a male intruder is present, a mother will often fight. Hansen and Ferreira (1986) injected bicuculline into the amygdalae of female rats with offspring and found that such "protective" aggression was much reduced.

Repeated stimulation of the amygdala appears to cause opposite effects to those of a lesion. Pinel, Treit and Rovner (1977) assessed aggression in terms of resistance to capture and reactivity to a tail tap, and found that repeated kindling (electrical stimulation) of the amygdala produced increases in aggressive responses to both forms of stimulation.

2.3 - SOCIAL RANK AND INTERACTION.

Rosvold et al. (1954) observed amygdalectomised primates in a natural social group, and found that the lesioned animals dropped rapidly in social rank to the bottom of their dominance hierarchies. Such animals become solitary, and frequently victimised and "beaten up". There is no data on social standing in rats to my knowledge, but lateral nucleus lesions in hamsters do result in a decline in rank, and similar results have been seen in lizards, dogs, and monkeys. Dogs can be seen to drop in dominance rank when it comes to competing for bones (Fuller et al. 1957).

The amygdala appears to influence the amount and type of interaction that an animal engages in. Rats with lateral nucleus lesions interact less with conspecifics, whereas septal lesions actually increase "sociability" (Jonason & Enloe, 1971). Injections of testosterone into the amygdalae of neonatal female rats "masculinise" their styles of play as pups; intracellular androgen receptors may well account for this (Meaney & McEwan, 1986).

2.4 - REPRODUCTIVE BEHAVIOUR.

Under the rubric of "reproductive behaviour", copulatory and maternal behaviour are the two main headings. Harris and Sachs (1975) lesioned the cortical and medial nuclei of male rats and found that this abolished copulatory behaviour, whereas basolateral lesions appeared to potentiate it. One behaviour that has been intensively studied is "lordosis" - a concave arching of the back which is a sexual response in rats. Masco and Carrer (1980) performed both lesion and stimulation manipulations, finding that anterior cortical and medial lesions decreased lordosis, and conversely anterior cortical stimulation increased it, posterior lateral stimulation attenuating it. Chateau and Aron (1988) confirm that posterior lateral lesions increase receptivity, lending validity to the methodological premise that lesion studies should opposite results to the stimulation of a particular structure. The role of the corticomedial division of the amygdala is again confirmed in McGregor and Herbert's (1992) experiment, who found that corticomedial lesions severely affected male copulatory behaviour, but basolateral lesions did not.

Maternal behaviour appears to increase after amygdaloid lesions, which Takahashi and Gladstone (1988) attribute quite plausibly to a reduction in fear towards newborn pups.

Electrophysiological recording studies have produced intriguing results, though no studies have yet been performed in the rat relating to social and emotional behaviour. Rolls (1981) discovered that many neurons in the macaque amygdala respond specifically to other macaque faces, and Kling et al. (1987) have studied the responses evoked to various warning vocalisations in squirrel monkeys. Both of these studies confirm the amygdala's role in processing affectively significant social stimuli.

2.5 - SPONTANEOUS BEHAVIOUR.

As long as the rat amygdala has been studied, researchers have investigated the effects of amygdalectomy on feeding, drinking, movement and other such simple behaviours. These results must be treated with caution, however, as radiofrequency, aspirative and electrolytic lesions were used which have been shown to damage fibres of passage[3] (Dunn & Everitt, 1988), particularly those associated with taste. This can have major behavioural effects (i.e. abolishing conditioned taste aversions - Dunn & Everitt, 1988) and means that data on spontaneous feeding must be handled sceptically. There may well be other "accidental" results of non-neurotoxic lesion methods, so I intend

[3]for a more detailed discussion, see the introduction to the "behavioural tasks" section

to omit studies that involve these techniques completely, along with those that utilise kainic acid (reported to cause "distant" neural damage).

Lorenzini et al. (1991) set out explicitly to investigate the effects of ibotenic acid lesions on spontaneous behaviours. Infusion of the acid into the basolateral amygdala caused no change in the level of feeding behaviours, though there was a shift towards feeding in the light (laboratory "daytime"). Drinking was reduced both day and night for a period of around three days, after which it was normal. Dunn and Everitt (1988) confirm this finding, contrasting it with a more chronic hypodipsia that resulted from electrolytic lesions (water intake was reduced for all six days of the experiment.) There was a less transient increase in exploration (measured using a multiple Y-maze), and locomotion also went up, the bulk of this increase being in the "dark" hours of a 24 hour cycle.

The increment in movement represented an accentuation of behaviours that were already performed, rather than an arbitrary increase in previously unobserved behaviours. Lorenzini et al. (1991) conclude that the basolateral amygdala has an inhibitory effect on locomotion and approach to novelty (one is reminded of Richard Gregory's "hiss inhibitor").

There is a great deal of data available relating to changes in food preference and gustatory neophobia in rats after amygdala damage (preference; Roll & Rolls, 1973, neophobia; Sutherland & McDonald, 1990). Such studies generally find altered preferences and reduced neophobia, but they used non-neurotoxic lesioning techniques, and are therefore flawed. Dunn and Everitt (1988) investigated gustatory neophobia using ibotenic acid lesions, and found that it was reduced, as with electrolytic lesions (a kind of "neophilia"). A parallel experiment was performed by Borsini and Rolls (1984) in which noradrenaline injected into the basolateral amygdala increased the amount of time spent eating familiar food in a preference test, (a kind of "double *ass*ociation"). Similar results of disrupted food preference have been reported in monkeys by Baylis and Gaffan (1991).

3 - BEHAVIOURAL TASKS AND REGULATED BEHAVIOURS

3.11 - INTRODUCTION AND COMPARISON OF LESION METHODS

When attempting to fathom the precise function of a brain structure, a favoured technique is the lesion method. This involves destroying the structure in question in a group of animals, and subsequently attempting to design a laboratory task that lesioned animals are clearly bad at compared to controls. An intuitive attempt is then made to grasp the processes involved in this task, and the result of this thinking is put forward as one of the functions of the relevant structure. This is the approach, peppered with the odd stimulation/infusion experiment, that has been taken in the vast majority of experiments that attempt to investigate the function of the amygdala, and the research reported here is firmly in this tradition.

This research is centered around the investigation of reward-related processes in the rat, and so I will only submit a brief review of the aversive conditioning literature. Also, I will give most weight to studies that use neurotoxic lesioning methods (the reasons for which I will discuss shortly). This section will commence with a discussion of lesion-producing techniques, and set up context by describing one of the principal theoretical approaches to amygdala function. After discussing the bulk of appetetive and aversive literature, I will include a separate section on discrimination learning to provide in-depth coverage of the background to this research

Traditionally, the favoured methods of making lesions have been aspiration, electrolysis and radiofrequency. Aspiration lesions are a commendably direct approach, consisting simply of sucking tissue out of an animal's brain. Electrolytic and radiofrequency lesions are closely related, the former using direct electric current to destroy cells, the latter using alternating current. The drawback to all three of these methods is that they not only destroy cell bodies in the area of lesion production, but they also kill any axons that may be passing through the lesion area but having no functional connection with it. The behavioural effects associated with the lesion could therefore be misleading, possibly due to damage to fibres of passage.

This is not a fine-grain methodological quibble, particularly in amygdala research. One of the most replicated findinds of amygdala research before 1988 was that amygdala lesions impaired learning of a conditioned taste aversion (CTA, i.e. Rolls & Rolls, 1973.) However, Dunn and Everitt

(1988) compared the effects of electrolytic and ibotenic acid[4] lesions on CTA, and they concluded (with the aid of a retrograde tracer experiment) that it was the destruction of insula-brainstem/hypothalamus fibres that ran through the amygdala that abolished CTA, not the amygdala itself. This result was replicated by Cahill & McGough in 1990.

The implications of Dunn and Everitt's findings are obvious. If experimenters use non-neurotoxic methods to lesion the amygdala, then there must be a very clear rationale for doing so. The research reported here uses N-methyl D-aspartate (NMDA), the favoured toxin of most contemporary experimenters, which acts at glutamate receptors to kill cell bodies.

3.11 - A THEORETICAL NOTE

Before starting my survey of the literature, it is worth noting the general theory of amygdala function that has been uppermost in researchers' minds for approximately the last forty years, which can be termed the "stimulus-reward association" theory. Weiskrantz (1956) proposed that the amygdala was the site at which sensory data become invested with their affective valencies. A more modern version of this idea is that the amygdala is involved in "behavioural tasks that require asscociations of neutral stimuli with incentive stimuli" (McDonald & White, 1993). These theories have arisen out of the need to explain the paradoxical findings that amygdalectomised animals still find food rewarding and are ostensibly "normal" in visual capacities, but show distinct deficits at learning visual discrimination tasks in which they have to associate a visual stimulus with a food reinforcer. David Gaffan's theory is the most detailed attempt at making sense of this. He proposes that the amygdala is involved in a particular class of stimulus-reward associations where a discrete stimulus is associated with its intrinsic reward value. Gaffan provides impressive and ingenious experimental support for this idea, and even though his work is exclusively on monkeys, his theory is so compelling as to merit a serious attempt to replicate it in the rat. I will deal with his theory and others in more detail in section 3.32, and the importance of this theoretical approach for the field in general and in particular for the research reported here will become apparent.

[4] a fibre-sparing neurotoxin

3.2 -STUDIES INVOLVING PUNISHMENT/AVERSIVE CONDITIONING.

McDonald and White (1993) have devised a useful list of aversive tasks/behaviours that amygdalectomised rats perform poorly on (though they inadvisably include conditioned taste aversion and gustatory neophobia.) I will loosely follow this structure in this section, looking in turn at fear potentiated startle, avoidance tasks, acquisition of conditioned emotional responses/autonomic conditioning/conditioned reaction to threat, and neophobic responses. A brief coverage of these areas will hopefully illustrate some examples of the kind of deficit encountered after amygdala damage, without including irrelevant detail. The assumption that any deficit in aversive conditioning will apply in an appetetive setting, however, is not safe, particularly in the light of Cahill and McGough's (1992) findings (see below).

3.21 - FEAR-POTENTIATED STARTLE

The principal work in this area has been performed by Davis and colleagues. The fear-potentiated startle paradigm is hearteningly simple; a UCS is paired with an aversive stimulus (i.e. a footshock.) Then, another aversive stimulus is presented (i.e. a loud noise) with or without the CS. If the CS is present, then the normal startle response (to the noise) will be augmented. Hitchcock and Davis (1986) lesioned the central nucleus of the amygdala, and compared the effects of this with bilateral transections of the cerebellar peduncles or bilateral lesions of the red nucleus. Only central nucleus lesions blocked potentiated startle (using the precise paradigm described above), and did so completely. Miserendino et al. (1990) paralleled this result, infusing AP5 (an NMDA antagonist) into the amygdalas of a number of rats, and found a dose-dependant blockade of startle potentiation. Since NMDA is clearly implicated in long-term potentiation (LTP) and learning in general, this implies a likely amygdaloid involvement.

On the basis of these and other experiments, Davis (1992) conludes that the central nucleus of the amygdala "may represent a central system involved in both the expression and acquisition of conditioned fear." His qualification that the amygdala may represent "a" central system in fear was clairvoyant; Kim and Davis (1993) found that amygdala lesions blocked acquisition and expression of

fear-potentiated startle (even with extensive training), but did *not* prevent reacquisition of the response. Evidently some other brain system is capable of providing a substrate for the potentiated startle response.

3.22 - AVOIDANCE TASKS

This type of task may be split into both passive and active forms. Active avoidance of a negative stimulus is self-explanatory, passive avoidance less so. A common example of a passive avoidance paradigm is the "step-down" task. An animal is placed on a platform surrounded by a wire mesh that can be walked on but can deliver a footshock. Once the animal is habituated to the platform and the mesh floor, the floor is electrified, and the animal has to learn *not* to step down onto it - avoidance of punishment by the suppression of a response.

This very paradigm was used by Dunn and Everitt (1988) in their series of experiments comparing the effects of ibotenic acid and electrolytic amygdala lesions on behaviour. Traditionally, amygdala lesions have consistently been shown to have an effect on passive avoidance learning (i.e. Pellegrino, 1968); Dunn and Everitt replicated this finding with respect to the retention of such behaviours, showing that the behavioural effects of amygdalectomy in this case are not due to the destruction of fibres of passage.

Cahill and McGough (1990) have investigated active avoidance in animals that have received NMDA lesions to the amygdala (large but subtotal; sometimes include central nucleus). Thirsty animals were placed in the "start" arm of a Y-maze, and allowed to approach a water dipper and drink freeely for the first two days of the experiment. On the third day, the rats received a footshock when they began to drink. The amount of time that animals took to approach the dipper on day four (the "latency to drink") was compared for lesion animals and controls, and amygdalectomised rats showed clearly lower latency to drink, whilst not differing in sensitivity fo footshock (measured by a flinch test). Cahill and McGough contrast the effects of such aversive learning with other appetitive tasks that they employed, and concluded that amygdala lesions differentially affect learning of appetitive and aversive tasks. This is a highly significant proposal, which they back up with a review of past literature and neurochemical evidence; their implication is that the role of the amygdala in

appetitive tasks is minimal compared to certain highly arousing, aversive tasks. This proposal is, of course, of major importance to this review, as it relegates appetitive amygdala research to a minor area of relatively peripheral interest compared to the weightier results that can be found elsewhere. Examining it critically, however, it is not clear whether Cahill and McGough are sidelining appetetive research or research on minimally arousing tasks; Everitt et al.'s (1989a) work on second order conditioning to sex is, of course, both.

An early experiment on avoidance learning after amygdala damage was performed by Robinson (1963). She found the characteristic deficits in active avoidance, but also noted an *increase* in the number of fear responses displayed by amygdalectomised rats. Finding a positive correlation between number of crouching responses and latency in avoidance responding, she drew the intriguing conclusion that the avoidance deficit was actually caused by the amygdalectomised rats being *more* afraid than their counterparts. This possibility casts a different light on such avoidance deficits, particularly as they had previously been attributed to hypoemotionality.

3.23 - ACQUISITION OF CONDITIONED EMOTIONAL RESPONSES

Selden et al. (1991) investigated fear conditioning in rats to contextual and explicit cues, using apparatus cues as contextual ("conditioned cue preference", CCP) and a clicking noise as an explicit stimulus. Footshock was the unconditioned stimulus. They performed two neural manipulations - lesioning the basolateral amygdala with quinolinic acid, or infusing 6-hydroxydopamine (6-OHDA) which depletes noradrenaline and dopamine. Both of the "surgical" groups of animals were "severely attenuated" in conditioning to the explicit "click" cue, but CCP was unaffected. Reverse results were found for the hippocampus. This result is interesting and valid on its own, but particularly so when compared to McDonald and White's (1993) finding that amygdala (lateral/basolateral) lesions impair acquisition of an *appetitive* CCP, and hippocampal lesions leave it untouched (also White & McDonald, 1993, see appetitive section.)

LeDoux et al. (1990) investigated the conditioning of autonomic responses to an aversive stimulus. Using a sound as a CS and a footshock as an UCS, they observed "freezing" responses and arterial pressure upon presentation of the CS. Lateral nucleus lesions (electrolytic) interfered with

both the behavioural and the autonomic measures of "fear", though they had no effect when the cs and ucs were paired randomly; this implies a specific role in stimulus-response learning.

A slightly different approach was taken by Blanchard & Blanchard (1972). Instead of investigating the acquisition of conditioned emotional responses, they demonstrated that lesions (albeit electrolytic ones) could abolish conditioned reactions that were already present. Although neurotoxic lesions are preferable, Blanchard & Blanchard's results seem in line with those described above, whilst also having the benefit of using ethologically relevant stimuli, so I shall cite them. Both total and corticomedial lesions produced reduced freezing to the presence of a (sedated) cat or an approaching shock prod (which was meant to simulate a predator). The lesioned rats were markedly unafraid, one even daring to climb onto the cat's back and nibble its ear. The cat gave this individual a good shaking and dropped him, whereupon he immediately climbed back again. Again, these results can be contrasted with those of Robinson (1963, see above), who found that amygdalectomised animals showed more fear responses (in terms of behaviours such as defecating, crouching, freezing) than controls.

3.24 - NEOPHOBIA.

A consistent result of early investigations was that amygdalectomised rats showed reduced neophobia generally, and specifically with respect to food (i.e. Rolls & Rolls, 1973). Sutherland & McDonald have replicated this as recently as 1990, but still using electrolytic lesions. Dunn & Everitt (1988) used ibotenic acid, and showed that the issue was rather more complex. Lesioned animals ate more of a novel food in a novel environment than controls, thus apparently supporting previous findings. In contrast to this, these animals showed no attenuation of response to a novel saccharine solution (which had previously been water) which was part of their CTA experiment, which would indicate intact neophobia. Dunn & Everitt conclude that this could be grounds on which to state that there must be more than one type of "novelty"; amygdala lesions may attenuate the effects of a novel environment, but not of a novel taste. It would be tempting to support this conjecture with recent evidence on the abolition of CCP to environmental cues (McDonald & White, 1993).

3.3 - STUDIES INVOLVING REWARD/APPETITIVE CONDITIONING

I intend in this section to be rather more "in-depth" than previously, as the studies reviewed here are the most relevant to the research at hand. Loosely following McDonald & White's scheme, I will consider tasks that involve differences in the magnitude of reward, and "conditioned reward tasks in which previously neutral stimuli are associated with stimuli that elicit approach." Under the second heading, I will include Gaffan et al.'s work (primates, but relevant), along with that of the Cambridge group (i.e. Robbins, Everitt, Burns). Gallagher et al.'s work does not fall easily into this classification, offering provocative insights into the complexity of "simple conditioning", and I will deal with this separately. In a concluding section, I will review and analyse all of the experiments to date that have investigated the effects of amygdalectomy on free (operant) appetetive discrimination tasks, and attempt to make sense of this contradictory literature and isolate the factors that make it so equivocal.

3.31 - TASKS INVOLVING DIFFERENT MAGNITUDES OF REWARD

A number of experiments support the thesis that the amygdala is involved in the successful performance of tasks that require a discrimination to be made between larger and smaller amounts of reward, and this is a principal component of the main study reported in this thesis. The first study that brought this function to light was performed in 1970 by Kemble and Beckman. Response latencies on a runway were measured, and amygdalectomised rats demonstrated an initial increase in latencies above controls which disappeared with training. When they manipulated the reinforcement schedule, the investigators found that the amygdalectomised rats were less responsive to such changes than controls; when the reinforcer was switched to extinction, amygdalectomised animals perseverated with low latencies longer than controls. Kemble and Beckman attributed these results quite plausibly to "response perseveration."

Henke et al. (1972) and Henke and Maxwell (1973) shifted the focus from perseveration to magnitude of reward, analysing behavioural contrast and the frustration effect respectively. Henke et al. employed a runway and two different running conditions (in the light/dark). When one of these components was switched to extinction, rats showed decreased response latencies in the other

component - this being "behavioural contrast." Amygdalectomised animals showed a preference for the reinforced component, but not the large increase that signals "behavioural contrast." This fits in with Kemble and Beckmans's response perseveration theory, but this idea is contradicted by Henke and Maxwells' (1973) results. The "frustration effect " is the temporary increase in responsiveness seen when a component is changed to extinction. Using a double runway, Henke and Maxwell found that amygdalectomised rats did *not* show the frustration effect; this supercedes Kemble and Beckman's finding of decreased latency when a component was changed to extinction (compared with controls). Thus amygdaloid damage appeared to produce a general problem with magnitude of reward distinctions, a result now regarded as having a reasonable experimental pedigree.

In yet another runway experiment in 1980, Goomas and Steele studied the collapse effect. If one group of rats is given a large reward to run for (12 pellets) and the other a small one (2 pellets) the group with the large reward will run faster initially, but will soon be "caught up" with by the "small reward" group. This is termed the "collapse effect", and happens sooner in amygdalectomised animals than in controls. Goomas and Steele added a brief delay into their reinforcement schedule; the brief increase in performance typically seen comes later in animals with amygdala damage, and the ensuing decrement in performance is less for them.

The first study to break away from runway methods was that of Kesner et al. (1989). The apparatus used was an 8-arm radial maze in which some arms contained seven pellets, and others only one pellet. Kesner et al. contrasted the effects of central and basolateral nucleus damage on this task, finding that only central lesions resulted in a performance deficit. Methodological considerations prevent us from accepting this result as revealed truth, however; the investigators used a rather small number of subjects[1], and lesions often affected the caudate-putamen and (more critically) invaded the optic tract.

Almost simultaneously, Peinado-Manzano (1989) published a study on learning a T-maze magnitude of reward (7 vs 1 pellets) visual discrimination task. She demonstrated that central and lateral amygdala lesions (kainic acid) both disrupted the acquisition, but not the retention of this task.

[1]central lesion n=7, basolateral n=5, no shams.

Lateral nucleus lesions proved to be more destructive, possibly due to this nucleus being a major site of convergence for sensory inputs.

Kentridge et al. (1991) included a magnitude of reward element in their T-maze experiments, but the implications of their findings are unclear for the issue of reward size; amygdalectomised animals were impaired both at 6 vs 0 and 5 vs 1 pellet conditions.

The one drawback with the lesion research up to this point was that all of the lesions were made before the shift in reward magnitude. It is possible, therefore, that the magnitude of reward problems could have been caused by a nonspecific performance deficit induced by the lesion. To settle this issue, Salinas, Packard and McGaugh (1994) performed a study in which they injected either lidocaine or a buffer solution into a group of rats, some of which had just undergone a magnitude of reward shift in a runway task.

The inactivation of the amygdala after the shift attenuated the response to it. Both groups of "shifted" animals (lidocaine injected and sham) showed the normal response initially to the reward shift (an increase in runway latency); when the amygdala was subsequently inactivated, the lidocaine group showed a significant decrease in runway latencies compared to the buffer injected animals. Salinas et al. suggest that the amygdala is the site at which memory for the aversiveness of the downward shift in reward magnitude was stored, and connect this hypothesis with other evidence on the effect of amygdalectomy on aversive conditioning and learning.

CONCLUSION

In summary, evidence points towards amygdaloid involvement in processes related to the magnitude of reward. This idea has been pursued using a number of behavioural tests - runway, radial maze, T-maze - but it does not yet appear to be possible to refine our description of the exact nature of the deficit beyond the vague label of "magnitude of reward problems". Salinas et al.'s proposal that the issue at stake is one of aversive learning can usefully explain all of the results found in the runway experiments, but leaves to the imagination what is happening in the radial maze/T-maze experiments described above. One further question is how much of a difference in magnitude of reward does there have to be before amygdalectomised animals show a deficit.

3.32 - THE ASSOCIATION OF NEUTRAL STIMULI WITH THOSE THAT ELICIT APPROACH: THE WORK OF DAVID GAFFAN etal.

David Gaffan has formulated the most coherent theory of amygdala function in the monkey that is available. A proposal of equivalent sophistication is not yet available in the rat, so I have no hesitation in looking to the monkey data to provide ideas that may apply across species.

Gaffan observes that amygdalectomised monkeys are relatively indifferent to the sight of rewarding or aversive stimuli - for example to a banana, or conversely a capture net. An intuitive explanation of this phenomena might imply that these animals have difficulties forming stimulus reward/punishment associations, but the fact that many food-motivated tasks can be performed quite normally gives the lie to this explanation.

In 1987, Gaffan and Harrison performed an experiment in which they tested the ability if monkeys to acquire a discrimination to an auditory secondary reinforcer; amygdalectomised monkeys were very poor at doing so. This appears to support the notion that the amygdala is crucial in forging connections between certain stimuli and their reward properties. In Mishkin and Petri's experiment (1984), however, lesioned monkeys perform normally on the Wisconsin General Test Apparatus (WGTA) in simple two-choice visual discrimination learning. It is hard to envisage this task being performed without stimulus-reward associations . Gaffan's theorising is an attempt to make sense of these apparently paradoxical results.

Gaffan rejects the idea that there is only one way for a monkey to learn a task. It is conventionally understood that monkeys perform as they do due to the associations that they have between stimuli and rewards. Gaffan proposes a second possible strategy, that monkeys merely learn a rule which generally leads to them getting food, for example win-stay, lose-shift or something as simple as "pushing aside an object often reveals food underneath." Post-amygdalectomy, perhaps animals lose the precise association between the sight of a particular food object and its taste, but recall the rule that reaching out and touching stimuli is often beneficial.

The analysis is deepened as Gaffan distinguishes three aspects of a "reward event" from a monkey's point of view. Component 1 is the intrinsic, unlearned reinforcing property of a piece of food - its taste in the animals' mouth, the animal's feeling of being "full." The visual appearance of the

reinforcer (i.e. a banana) - which has no rewarding value of its own - is component 2, and the emotion evoked by the sight of the food is seen as component 3. The fundamental hypothesis is that amygdala damage disrupts the connection between components 1 and 2 - and thereby makes component 3 impossible, explaining the hypoemotionality to the sight of food.

This scheme can be used to explain the defecits in auditory secondary reinforcement. Gaffan and Harrison (1991) have demonstrated that it is hard for monkeys to associate visual stimuli with sounds, *unless* the sounds are emotionally different, i.e. they differentially predict food reward. The auditory secondary reinforcer's visual associability therefore stems from its connection with the food reward, and this is disrupted after amygdalectomy - so no learning. Associating visual stimuli with other visual stimuli is, in comparison, easy for monkeys, irrespective of their "emotional similarity." Discrimination learning in a situation where a visual secondary reinforcer is spatially and temporally close to the discriminanda can, then, proceed by one of two routes:

(1) A visual-visual association between the visual discriminanda and the component 2 (visual) properties of the visual secondary reinforcer. Or,

(2) an association between the discriminanda and the emotional response evoked by the secondary reinforcer.

Gaffan argues that only route (2) is disrupted after amygdalectomy, and indeed visual learning for a local secondary reinforcer is much better after amygdalectomy than for an auditory secondary reinforcer.

We can conceptualise standard discrimination learning in these terms. The discriminanda is almost like a secondary reinforcer, forming a visual-visual association with the sight of the food reward. Gaffan & Bolton (1983) have clearly demonstrated that monkeys are capable of forming such associations. As long as the reward is near the discriminanda, animals should be able to discriminate normally. If they are spatially/temporally distant, however, learning should be disrupted. As performance on the WGTA depends upon the displacement of an object above a well containing a peanut, it is unsurprising that amygdalectomised animals can perform this task.

If this theory is true, it should result in the rather bizarre consequence of monkeys continuing to perform discrimination tasks even if they are unrewarded, as Gaffan does not really account for why the monkey would *wish* to form such visual-visual associations. This counterintuitive proposal is

almost certainly not one that David Gaffan would wish to support, but it is a necessary consequence if one takes his theory as written. Stranger still, there appears to be even some support for this hypothesis in Kemble and Beckman's (1970) experiment, in which they showed that when reinforcement schedules were switched to extinction, amygdalectomised rats kept trversing the runway faster and for longer than controls.

A number of attempts have been made to explicitly support Gaffan's hypothesis (not my extension of it). Notably, Gaffan and Murray (1990) arranged a discrimination task in which no immediate visual feedback was given after a monkey made a response, and the food reward was delivered into a hopper placed away from the screen. The possibility of visual-visual associations being made between the discriminanda and the food was thus minimised, and the amygdalectomised animals did indeed show a deficit. In Baylis & Gaffan's (1991) experiment, amygdalectomised animals were tested on a two-choice discrimination between objects that they could suck to obtain a fruit-juice reward. As the food enters the mouth directly, visual-visual associations between the discriminanda and the sight of the food reward were impossible. Amygdalectomised animals were severely impaired at this task, and if one places these results alongside previous demonstrations of sparing of WGTA performance, it provides appreciable support for Gaffan's theory. There is one non-replication of Gaffan's results (Overman et al., 1990), but this is clearly accounted for by Gaffan in his 1994 experiment on picture discrimination learning.

SUMMARY:

Gaffan claims that amygdalectomy in monkeys disrupts the association between the reward value of the reinforcer and its visual properties. This produces the problems that can be seen ion acquiring secondary reinforcers, yet explains the sparing seen in discrimination tasks where discriminanda and reward are spatio-temporally close. His theory is rather weaker at explaining why animals would *want* to form the visual-visual associations that allow some forms of discrimination learning.

3.33 - APPETITIVE SECONDARY REINFORCEMENT, AND AMYGDALA-STRIATAL INTERACTIONS - THE WORK OF EVERITT, ROBBINS et al.

Following a discussion of D. Gaffan et al.'s work, it is appropriate to look at the research of Everitt et al.. This group has also emphasised the role of the amygdala in mediating secondary reinforcement, but their work has been performed exclusively on rats. The targets of their manipulations have been both the amygdala (always the basolateral area) and the ventral striatum (principally nucleus accumbens), as they aimed to investigate the functional significance of the closely related termination of amygdaloid efferents and mesolimbic dopaminergic neurons in the ventral striatum. The A10 dopaminergic neurons have been studied quite extensively, and appear to play an appreciable role in reward/incentive processes; consequently, Everitt et al.'s work is a study of appetitive secondary reinforcement in rats. This section of the review will summarise and number of this group's studies - Everitt et al. (1989a), (1989b), (1991), Cador et al. (1989), Burns et al. (1993) - drawing heavily on Everitt and Robbins (1992)

TESTING PARADIGMS

Everitt et al. have devised three separate behavioural tasks with which to assess the effects of their various neural manipulations.

(1) Acquisition of a new response.

An arbitrary stimulus is paired with a reward (e.g. water for a thirsty rat). The rat is then presented with two levers, one of which will cause the conditioned stimulus to appear when pressed. This test is intended to assess the power of the secondary reinforcer over behaviour (essentially, its reward properties).

(2) Conditioned place preference (CPP).

The rat is allowed to explore two distinctive environments, one of which consistently contains food, the other nothing. The rat is then allowed to choose which of the two environments to enter.

(3) Second order schedule of reinforcement.

Male rats are allowed to interact sexually with a oestrous female in the presence of an arbitrary stimulus (a light, for example), which becomes a CS. Presentation of the CS is then allowed on a fixed ratio basis to lever pressing, followed by presentation of the female after a fixed interval. The rate of

responding determines what reinforcing powers the CS has acquired during the initial "association" stage.

TASK (1); neural manipulations and their results.

Infusion of cocaine, d-amphetamine or dopamine (generally, any dopaminergic agonist) into the nucleus accumbens potentiate responding on the correct lever at a level that depends on drug dosage. Depletion of dopamine from the ventral striatum using 6-OHDA prevents this effect. Lesions of the basolateral amygdala with NMDA or quinolinic acid, however, lead to a significant reduction in the ratio of CR:NCR (S+:S-) responses. Cador et al (1989) noted that this reduction was due to a decrease in responding on the CR lever, but this contrasts with Burns et al.'s (1993) observation of an increase in responses on the NCR lever. Either way, the lesion is clearly reducing the salience and efficacy of the conditioned reinforcer.

TASK (2)

Quinolinic acid lesions of the amygdala and quisqualic acid lesions of the ventral striatum both abolished a preoperative CPP. Everitt et al. (1991) also performed crossed lesion of the amygdala and ventral striatum, and compared them with unilateral lesions of either single structure. The crossed lesions did attenuate CPP, though rather less than bilateral lesions of either structure alone. Unilateral lesions of either structure had no effect on CPP.

These results, especially from the crossed lesions, appear to support the idea that expression of a CPP requires the serial interaction of the amygdala and ventral striatum. Everitt et al. (1991) also induced lesions of the ventromedial and dorsolateral caudate/putamen in order to assess these structures as other possible candidates for a role in CPP; dorsolateral lesions had no effect, but ventromedial lesions produced attenuation of a similar magnitude to ventral striatal (nucleus accumbens) lesions. Measuring a number of other behavioural indices (e.g. locomotion, water consumption) and finding them equal allowed Everitt et al. to rule out other spurious deficits as possible causes for the deterioration for CPP observed.

TASK (3)

The number of lever-press responses recorded after amygdala damage was significantly lower than controls (Everitt et al., 1989a). When presented with a female in oestrus, however, the male rats displayed no difference in copulatory behaviour from sham animals. Intra-accumbens infusion of d-

amphetamine increased lever-pressing to the extent that lesioned animals did not differ from controls, and made initiation of copulatory behaviour more rapid in both groups.

One intriguing observation made by Everitt et al. (1989) was that when the CS (light) was removed from the schedule, the performance of control animals dropped drastically - to a level lower than that of the amygdalectomised animals. Everitt et al forward the possible explanation that animals with amygdala lesions are being comparatively unresponsive to a shift in apparent magnitude of reward.

CONCLUSIONS:

Everitt and Robbins (1992) conclude that their results offer support to Gaffan and Harrison's (1987) assertion that the basolateral amygdala is important in mediating secondary reinforcement. They point out that although results appear homogeneous, tests may differ radically in their demands upon the animal, and it is also noted that different reinforcers are used in each test (water, sucrose, sex, respectively).

The exact nature of the deficit caused by basolateral amygdala lesions remains undetermined. Everitt and Robbins note that the deficits seen could be caused by disruption of CR - UCR linkages, or perhaps UCR - instrumental response associations. Also, the age-old classical/instrumental conditioning dichotomy blurs in situations such as the CPP procedure. Is the rat entering the correct (S+) environment through instrumental "choice", or through a Pavlovian "approach tendency"? Much thought is needed to clarify this and other such issues, as well as experimentation.

3.34 - THE CENTRAL NUCLEUS AND "SIMPLE CONDITIONING" - THE WORK OF HOLLAND, GALLAGHER et al.

The mechanism of classical conditioning is so fundamental in psychology as to be taken for granted. Holland and Gallagher's work has centered around the Pavlovian association of a CS with food, and on the basis of a number of experiments they conclude that this process is not nearly so straighforward as it is commonly held to be. Their work covers a number of areas, but I will concentrate here only on their distinction between CS- and US- generated behaviours.

Holland and Gallagher (1992) summarise a number of experiments in which they have detected and used the distinction between CS- and US- generated behaviours. The paradigmatic

situation used is of pellets being dispensed into a food tray, with the associated noise of a dispenser motor and a light being illuminated. CS-generated behaviours resemble the normal response to such cues as a light coming on , or the sound of a dispenser. A rat will rear when the light comes on, and orient towards it; when the dispenser noise is heard, the animals will exhibit a limited startle response. In the experimental paradigm used by Holland and Gallagher, the rats are exposed to such visual and auditory cues before conditioning, and habituate to them. When conditioning starts, however, the animals reacquire these orientation/startle behaviours, and in this new context they are referred to as CS-generated behaviours.

When auditory and visual cues have been consistently associated with food delivery, they come to elicit a different set of behaviours. These new CRs - unsurprisingly - closely resmble the normal response to food delivery. On presentation of visual/auditory cues the rat stands still with its head in the food tray, or possibly makes rapid head movements towards the food tray. These movements are highly dependant on the nature and form of the US, and would not occur in response to light/noise prior to conditioning. Holland and Gallagher (1992) refer to these behaviours as US-generated behaviours.

Gallagher et al. (1990) set out to investigate the role of amygdaloid central nucleus (CN) lesions on these two categories of behaviour. CN lesions had no effect on the initial (preconditioning) generation of rear and startle behaviours, but the lesions did impair the reacquisition of these behaviours during conditioning. US-generated behaviours were left intact by the lesion, and the sparing of preconditioning rear and startle behaviour rules out any possibility of a general motor/sensory deficit causing the later impairment.

This result is intriguing, and any theory pertaining to the amygdala's role in the perception of such stimuli as traylight and dispenser noise is highly relevant to this experiment (see section 5, with respect to stage 2). It is quite hard to determine what effect such a deficit would have on discrimination learning performance (as tested in experiment one) apart from a possible transient decrease in the time taken to perform the discrimination due to the absence of rear/startle behaviour.

3.35 - CONDITIONED PLACE/CUE PREFERENCE

Everitt et al. (1991) have demonstrated that basolateral amygdala lesions abolish appetitive conditioned place preferences. This result is supported by a number of investigators, and interestingly also highlights a further area of behaviour in which the amygdala may make differential contributions to the processing of appetitive and aversive stimuli (see Cahill and McGaugh,1990).

Conditioned place preference (CPP) or conditioned cue preference (CCP)[1] is tested in a manner that is fairly consistent between experimenters. Animals are allowed to explore two distinctive environments, and in one of these they consistently experience some form of reward or punishment. The same animals are then later given access to both environments, and the amount of time spent in each environment (or some other behavioural variable observed in each environment) is measured. In 1991, both Everitt et al. and Hiroi and White observed the abolition of an appetitive CPP following lateral/basolateral amygdala lesions. The incentive stimuli used in these experiments were food and subcutaneous injection of d-amphetamine, respectively. In 1993, McDonald and White replicated this effect (CCP to food), and contrasted it with the absence of a deficit after destruction of the hippocampus or dorsal striatum, which caused no deficit.

The amygdala appears to play no role, however, in the formation of aversive CCPs. Selden et al. (1991) found that amygdalectomised animals displayed normal conditioning to place/environment when one environment had been consistently paired with shock. This contrasted with the finding that hippocampectomised animals were severely retarded in choosing the "safe" (non-shock) environment from contextual cues. Sutherland and McDonald (1990) performed a slightly different experiment, involving the simple association of a single environment with shock; aversive conditioning was assessed by measuring an animal's amount of defecation when placed in that environment. Amygdalectomised animals showed elevated defecation when placed in the conditioned context after several pairings with shock, whereas hippocampectomised animals did not. Once again, an intact amygdala does not appear to be a prerequisite for acquiring aversive conditioning to contextual/environmental cues.

In summary, the amygdala appears to be necessary for conditioning to both contextual (CCP) and explicit (see section 3.33) appetitive cues, but not for contextual aversive cues. This appears to

[1] a title that implies less theoretical confidence as to the processes at work

contradict Cahill and McGaugh's weighting of the amygdala's importance with respect to reward/punishment somewhat, emphasising the amygdala's role in reward processes.

4 - THE EFFECT OF AMYGDALECTOMY ON FREE OPERANT DISCRIMINATION LEARNING

4.1 - INTRODUCTION TO DISCRIMINATION LEARNING AND REVIEW OF PREVIOUS EXPERIMENTS

A simple definition of discrimination learning runs as follows: an organism is reinforced for making a response in the presence of one stimulus (S+), but not in the presence of another. Discrimination learning can be divided into two forms, successive and simultaneous. If a rat is reinforced for pressing a lever when a light is on (S+), but not when it is off (S-), this is classified as a successive discrimination. The alternative to this is when S+ and S- are presented simultaneously, for example when both levers in a Skinner box are presented simultaneously, only one of which is S+.

The role of the amygdala in discrimination learning was first considered by Weiskrantz (1956). He did not explicitly test his monkeys on discrimination tasks, but stated that his evidence "appeared to rule out" any deficit. Schwartzbaum (1965) and Schwartzbaum and Poulos (1965) followed this suggestion up with tests of simple discrimination learning and generalisation, and also a study of reversal learning and learning set, finding no simple discrimination deficit, but a clear impairment at reversal learning. A number of monkey experiments over the years followed this issue up with little overall agreement. For example, Jones and Mishkin (1972) found deficits in both object and spatial discrimination, whereas Aggleton and Passingham (1981) found normal performance in their lateral and basolateral nucleus lesioned groups. Gaffan's theories (see section 3.32) have added a whole new level of sophistication to the topic, as well as positing a theory that has yet to be falsified (but see Overman et al. (1990) and Gaffan's (1994) reply).

As well as conducting some of the early research on monkeys, Schwartzbaum et al. (1964) initiated research into discrimination learning by amygdalectomised rats. Finding clear deficits, Schwartzbaum et al. concluded that experimental animals were having problems with response inhibition, or with "S- control of behaviour"; this resulted in the animals persevering with inappropriate responses, which radically depressed performance on a go-nogo tone discrimination task. Pellegrino (1968) attempted to replicate this result using a go-nogo *light* discrimination, but failed to do so although other experimental tasks indicated that response inhibition problems were present. He suggested that the use of a light discriminanda might explain his failure to replicate the

earlier result. Kemble and Beckman (1970) investigated a behaviour termed "vicarious trial error" (VTE), and used a position discrimination in a T-maze to do this. A VTE consists of an animal hesitating excessively or failing to choose which arm to run down in a T-maze, and the amygdalectomised animals displayed significantly more VTEs than controls (which does not seem consistent with a response inhibition deficit). Amygdalectomised animals also showed inferior discrimination and reversal learning performance compared to controls.

In 1974, Freeman and Kramarcy performed a clear replication of Schwartzbaum et al.'s (1964) go-nogo tone discrimination experiment finding that amygdalectomised animals performed poorly compared to shams and hippocampectomised rats. Again, Freeman and Kramarcy cited the notion of response inhibition in explaining their results. In contrast, Han and Livesey (1978) reported "no more perseveration or perseverance tendency" in their lesioned animals, and used a simultaneous brightness discrimination to assess their performance. Han and Livesey drew on Douglas and Pribram's (1966) theory of limbic system function in designing their experiment; in one condition, S+ stayed on after the response was made ("enhanced positive"), and in another S- stayed on ("enhanced negative"), and in the "non-enhanced" condition both stimuli disappeared simultaneously when a response was made. The "non-enhanced" condition is the most relevant in terms of compatibility with other studies, and amygdalectomised animals performed at a comparable level to hippocampectomised and sham subjects.

Eichenbaum et al. returned to go-nogo methodology in 1986, this time using olfactory cues as discriminanda. Despite the amygdala's heavy olfactory input, no differences in initial discrimination learning or reversal learning were found. This result is backed up by Slotnick and Kaneko (1981), who lesioned both MD and the olfactory input to the amygdala, only finding a go-nogo olfactory discrimination deficit in the MD animals. Yet another go-nogo experiment was performed by Peinado-Manzano in 1988, but unlike Pellegrino she did find a deficit in a bright/dim light discrimination. This leaves the issue of go-nogo tasks and their sensitivity to amygdala damage rather confused.

Kentridge et al. (1991) deployed a number of tasks aimed at taxing an animal's ability to associate stimuli with rewards. One of these was an object discrimination task in a Grice box. Two object discriminations were learned normally by amygdalectomised animals, but an impairment was

recorded for reversals 4 to 7. No effect was observed for reversals one to three, and this adds an intriguing slant to the issue of reversals; Kentridge et al.'s animals fail to acquire the facilitation of learning that normally occurs after several reversals.

This summary of research into discrimination learning and the amygdala has revealed no clear pattern of results. The following section may find some order in these experiments. I intend to attempt an in-depth examination of these studies, where appropriate using meta-analytic techniques, to try and isolate such specifics as: what is the overall conclusion of their research to date? which (if any) nuclei appear to be most important for discrimination learning? which styles of discrimination task are most sensitive to amygdala damage?

4.2 - META-ANALYSIS:

4.21 - A NOTE ON SAMPLING

A critical stage in any meta-analysis is the selection of studies to include in calculations. Current systems of research evaluation and publication emphasise studies with "positive results", which in this case would be showing a deficit after amygdala damage. The meta-analyst must face the possibility that a number of unpublished studies exist in which a "negative" result has been recorded (i.e. no postlesion deficit), and that by not including these the result of the analysis is biased. This is known as the "file drawer problem".

Superficially, there appears to have been little of this bias in the research reviewed, as four out of eight studies candidly report no post-lesion deficit. A statistical procedure exists, however, that can give an indication as to whether the overall significance of a group of studies could be threatened by unpublished research (Rosenthal, 1991, p261), and the results of this procedure will be reported in the course of the analysis. This will hopefully neutralise the danger of publication bias.

Another possible objection to the meta-analytic procedure used here stems from the nature of lesion research. The techniques demonstrated by Rosenthal (1984, 1991) all assume experimental situations in which the results could be influenced in any direction by a treatment (e.g. a drug could improve or worsen a patient's status). It could be argued that it is uncommon for a brain lesion to produce increments in task performance, and that therefore the results of the meta-analysis are biased towards showing an overall lesion effect when summating a number of studies. This objection is incorrect on two fronts - brain lesions can and do cause increments in performance, and this is also

true of amygdala lesions (see Weiskrantz, 1956, on extinction of avoidance learning, and section 5). Also, the "file drawer" calculations may help to allay fears here.

Kentridge et a. (1991) cite a study by Eleftheriou, Elias and Norman (1972) as germane to the issue of discrimination learning. This study is deliberately not included here for a number of reasons - first, it was performed on deermice; second, the task used resembles an avoidance paradigm (swimming to find an escape ladder); third, there is absolutely no description of histology apart from three photomicrographs, one of which shows a lesion in danger of invading the optic tract (fig 1, page 70, top section). This experiment is substantially different in both species and method to the others, and will therefore not be included.

Studies included in the analysis:

Table 1 (overleaf) is intended as a complement to appendix A, providing a brief summary of the studies that I am now going to compare and contrast.

4.22 - OVERALL CONCLUSIONS OF RESEARCH TO DATE ON INITIAL DISCRIMINATION LEARNING:

Superficially, it appears that the results are well split, as four studies show a deficit in performance, and four show no significant change. It is always possible, however, that studies showing no effect are displaying a type II error, and failing to show a significant result due to low subject numbers (low *n's*). Rosenthal (1984) cites the meta-analytic technique of adding weighted Z's[1] as a robust method of summating the p-values of studies so as to obtain an overall value; this technique should eliminate "power" problems by elevating overall n, and give the broad summary required. At the outset, it should be noted that there are no simplistic power problems present. On average, the studies showing a null result have higher n's (28.25) than those highlighting a deficit (19.25), though study (g) biases these figures somewhat.

METHOD: Accurate p-values were obtained by a number of methods. Wherever preceise F, t or other equivalent statistics were available, these were converted into p-values using the MINITAB statistical package. It should be noted that this package could not give accurate p-values of below p=9*10^-5; any

[1]called the Stouffer method by Mosteller and Bush (1954).

TABLE 1:

Study name and date	No. of animals in relevant comparison groups	Behavioural procedure	Lesion method	Does the lesion cause a deficit?
(a) Eichenbaum et al. (1986)	5 am, 5 control	Go/nogo task, discriminating odour	Electrolytic	No
(b) Freeman & Kramarcy (1974)	5 am, 10 control	Go/nogo task, discriminating tones	Electrolytic	Yes
(c) Han & Livesey (1977)	8 am, 30 controls	Brightness discrimination	Electrolytic	No
(d) Kemble & Beckman (1970)	11 am, 8 controls	T-maze spatial discrimination	Electrolytic	Yes
(e) Kentridge et al. (1991)	7 am, 7 controls	Object discrimination, Grice box	Ibotenic acid	No
(f) Peinado-Manzano (1988)	18 am, 12 controls	Go/nogo, discriminating brightness.	Ibotenic acid	Yes
(g) Pellegrino (1968)	30 am, 21 controls	Go/nogo, discriminating houselight on/off	Electrolytic	No
(h) Schwartzbaum et al.(1964)	7 am, 6 controls	Go/nogo, discriminating tones	Electrolytic	Yes

The result of this part of the analysis is conclusive. The overall conclusion is that amygdala damage does adversely affect discrimination learning/performance, but viewing the wide range of methodologies and lesions used this result is rather isolated without some more detailed analysis.

4.23 - ANALYSIS BY BEHAVIOURAL TASK:

A wide range of tasks and discriminanda have been used to assess the discrimination performance of amygdalectomised animals. An obvious question is - do any particular tasks record a deficit more consistently than others? The first comparison made here will be between simultaneous and successive discrimination tasks.

Five studies use successive discrimination tasks, all in go-nogo format (a, b, f, g and h). the p-values of thses can be combined and then compared with the p-values of the remaining three studies. P-values are combined using the Stouffer method of adding weighted z's (as above):

$$\text{Overall } Z = \frac{w_1Z_1 + w_2Z_2 \ldots w_8Z_8}{\sqrt{w_1^2 + w_2^2 \ldots w_8^2}}$$

The successive discrimination tasks summed to a z of 3.31 (p=0.0005), and the simultaneous yielded a z of 2.33 (p=0.01). Clearly, a larger deficit is found in successive tasks. Studies can also be combined by a different method to yield effect size measures (Pearson's r). The effect sizes tell a similar story, if anything showing the difference between the two styles of task to be greater. For successive tasks, r=0.608, simultaneous r=0.396. The method used to calculate these values was as follows:

(1) calculate effect size for each study , using:

$$r = \frac{Z}{\sqrt{N}}$$ (Rosenthal, 1984, p25).

(2) transform each r to z_r using Fischer's r to z transform

(3) Again, weighting by study size/10, calculate mean z_r:

$$\text{mean weighted } z_r = \frac{\Sigma w_j z_{rj}}{\Sigma w_j}$$

where w is the weighting of the study (n/10).

(4) reverse the r to z_r transform, yielding mean r for the five successive and three simultaneous studies.

All five successive discrimination studies use go-nogo methodologies. As noted above (section 4.1), the only consistency perceptible here is that both tone discrimination studies record a clear deficit.

The simultaneous discrimination studies are most relevant to Experiment one, the main study at hand. Studies c and e most closely resemble a Skinner box task, and both of them record no lesion effect. Study d uses a T-maze to stage a spatial discrimination (which is most relevant to experiment one) and *does* register an effect. No obvious prediction for Experiment one arises from these three studies. In the absence of a prior Skinner box experiment, it helps to note that when the p-values of all three simultaneous discrimination experiments are combined, the overall p-value produced is significant in the direction of a lesion effect ($p=0.01$), and this is probably the best conclusion to rely upon for predictions.

4.24 - ANALYSIS BY METHOD OF LESION PRODUCTION AND LESION PLACEMENT

Given the functional differentiation of the amygdaloid nuclei, it is quite plausible that the successful performance of a discrimination learning task is reliant on the intact functioning of a single nucleus or subgroup of nuclei. This section investigates this possibility, along with the question of whether particular methods of lesion production or extra-amygdaloid damage cause any significant functional effect.

Clarity is a genuine problem when splitting the results "by nucleus". Most of the lesions in the eight studies reported are not, of course, confined to a single amygdaloid nucleus, and generally include partial damage of various degrees to other nuclei. The attempt has yielded more negative than positive results.

Damage to the lateral nucleus appears to be irrelevant in terms of discrimination learning. Comparing studies that showed a lesion effect with those that did not, levels of lateral nucleus damage are almost identical between groups (two studies - complete ablation, one partial, one consistent ablation in 1/2 of Ss). An analysis of basal nucleus damage is less revealing. Various amounts of basal nucleus damage can be seen in both "lesion effect" and "no effect" studies, allowing no clear conclusion to be drawn. All of the other nuclei follow a similar pattern, with amounts of damage varying only slightly between "effect" and "no effect" groups, differences not being large enough to permit any strong conclusion to be made.

The function of the medial nucleus can be clarified a little further. Three out of the four studies that recorded a lesion effect involved no damage to the medial nucleus, clearly eliminating it from a role in discrimination learning. Overall, the mix of data and "messiness" of lesions force an unsatisfactory, inconclusive result to this part of the analysis.

Studies e and f both use ibotenic acid to induce their lesions, but record different results. In fact, the results differ significantly at $p=0.014$, indicating that the most powerful factors at work are probably lesion placement and task type (both of which differ between e and f) rather than method of lesion induction.

A final consideration is the amount of extra-amygdaloid damage sustained between studies a and h. Even in the earliest experiment of this set (Schwartzbaum et al. 1964), the authors suggest that caudate damage could be at least partly to blame for the deficit in discrimination learning that was found. Can any of the variance in subsequent results be attributed to such damage?

The provisional answer to this question appears to be "yes". The studies that record a lesion effect appear to display much more extraneous damage than the others.

Studies that record a lesion effect:

b - No histological description, reconstructions show extensive ventral piriform cortical damage.

d - Consistent ventral piriform and claustrum damage.

f - Very little extraneous damage. Possibly fractional caudate damage.

h - Consistent damage to the claustrum, ventral white matter, ventral putamen and globus pallidus.

Studies that do not record a lesion effect:

a - No extraneous damage described or apparent in reconstruction.

c - No extraneous damage described or apparent in reconstruction.

e - Reconstruction shows some cortical damage (medially).

g - No histological description given. Reconstruction (and method) suggests minimal extra-amygdaloid damage, possibly minor cortical/caudate damage.

No extra-amygdaloid area presents itself as particularly important here, and a simplistic attribution of discrimination deficits to extra-amygdaloid damage is obviously incorrect, as it would be inadequate to explain the results for experiment f. Also, it is simply possible that the studies involving the most extra-amygdaloid damage are also those with the largest lesions, and that lesion size is the most important variable. It is interesting to note that a recent reassessment of the on amygdaloid contribution to memory in monkeys has been sparked by evidence that the cortices adjacent to the

amygdala and hippocampus were performing function previously attributed to the amygdala (Zola-Morgan et al., 1989 a+b). Clearly with this parallel and the evidence presented here. the issue of extra-amygdaloid damage is not trivial.

4.25 - REVERSAL LEARNING

Kentridge et al. (1991) cite studies a, d, g and Eleftheriou et al. (1972) as addressing reversal learning, noting that a and g show not deficit and that d and Eleftheriou et al. do so. Following section 4.21, Eleftheriou et al.'s study is omitted, and can be replaced by Kentridge et al's study which does show a deficit in later reversal learning. Unfortunately, precise p-values can only be obtained from studies e and g, disallowing a full meta-analysis, and leaving two studies showing a lesion effect, and two not. It is possible that the results would be significant when summated, and also possible that they would be more clear if all of the experiments had extended to seven reversals, as study e notes that the lesion effect size increases in later reversal stages.

4.3 - CONCLUSION OF META-ANALYSIS ON INITIAL DISCRIMINATION LEARNING

A summary of the eight studies chosen for this analysis shows that their combined p-value indicates a significant lesion effect. Such a range of tasks and lesions were used in these studies, however, as to make this result uninteresting without more detailed analysis. This analysis showed that successive discrimination tasks were slightly more sensitive to amygdala damage than simultaneous tasks (effect size being around r=0.2 stronger), and that few conclusions could be drawn about the involvement of specific nuclei, apart from the elimination of the medial and lateral nuclei from a significant role. The use of neurotoxic (as opposed to electrolytic) lesioning methods appears to be secondary in importance to other factors, though there is a prima facie correlation between lesion effects and extra-amygdaloid damage. Such damage to surrounding cortices has proved important in the monkey and may be equally so here, although the simple confounding factor of lesion size has not been ruled out. A clear conclusion on reversal learning has not been reached, although it is evident that the differential results between reversals 1-3 and 4-7 discovered by Kentridge et al. (1991) will have to be taken into account when designing future experiments. To fully appreciate the amygdala's role in discrimination learning, it also appears that more theoretical insight into the processes involved in such learning and the differences between successive and simultaneous tasks will have to be attained.

5 - EXPERIMENT ONE: POSITION DISCRIMINATION.

Experiment 1 sets out to test three separate hypotheses. First, are amygdalectomised rats impaired at performing a position discrimination task? Second, do they show any decrement in performance when spurious secondary reinforcers are added to the task, so that its feedback for correct and incorrect responses appear (in terms of auditory/visual characteristics) the same? Third, can normal and amygdalectomised rats solve a discrimination in which the secondary reinforcers are equated as above, with the difference between the S+ and S- being the amount of reward (2 pellets vs 1 pellet.)

The overall conclusion of the review and meta-analysis was that amygdalectomised animals were in general impaired at discrimination tasks. The most relevant study to this experiment is Kemble and Beckman's (1970) investigation of position discrimination performance in a T-maze, where amygdalectomised animals were clearly impaired at both acquisition and reversals. Position discrimination in a Skinner box is a slightly different task, however, utilising egocentric rather than allocentric cues (for a discussion of this distinction, see O'Keefe and Nadel, 1978), and the question of whether Kemble and Beckman's results will replicate in these conditions is unanswered. It is predicted that a deficit will be found.

A further prediction is derived from Kentridge et al.'s (1991) study. Stage one of this experiment is designed with sufficient reversals to attempt to replicate their finding of a more severe reversal deficit in the reversals after reversal three. It is predicted that reversals 4 to 6 will show more of a lesion effect (i.e amygdalectomised animals inferior) than reversals 1,2 and 3.

In a Skinner box, a number of cues gain secondary reinforcing properties by becoming associated with the delivery of food reward. The noise of the pellet dispenser and illumination of the traylight just precede the arrival of the reward pellet in the food tray, and by virtue of this they can become secondary reinforcers. The question posed in stage 2 of the experiment is - what happens if the same secondary reinforcers come to be present after both correct and incorrect responses, making them indistiguishable apart from the presence/absence of reward pellets? Two bodies of data are relevant here. Everitt and Robbins (1992) conclude from a number of experiments that amygdalectomised animals are relatively insensitive to secondary reinforcers. From this position the prediction would be that sham animals would find the task harder due to the confusion of normal

cues, but that amygdalectomised animals would show no change in performance, as they were insensitive to these cues anyway.

A different prediction would be made from Gaffan's theory. Gaffan suggests that amygdalectomised monkeys perform discrimination tasks by forming visual-visual associations between spurious secondary cues and the visual presence of food reward. Having devised a number of tasks which minimise the ability of animals to make this kind of association, Gaffan has shown (Baylis & Gaffan, 1991, Gaffan & Murray, 1990) that the performance of amygdalectomised monkeys suffers in these conditions.

In stage II, as many of the secondary cues (traylight, dispenser noise) as possible have been made the same for both incorrect responses and correct responses, making it difficult for any animal to form the associations that would allow them (in Gaffan's theory) to perform the task normally. The clear prediction from Gaffan's theory would therefore be that amygdalectomised animals would show a deficit in performance compared to controls.

The predictions from Everitt & Robbins' and Gaffan's theories about stage II of this experiment contradict each other. As Everitt and Robbins' research has been performed on rats, however, it may be more applicable for this experiment. Also, differences in lesion style set the two sets of data apart; Gaffan's research involves aspiration lesions that generally destroy adjacent perirhinal and pyriform cortex, and the functions of these regions have not yet been accurately determined (although their functional significance appears considerable - see Zola-Morgan and Squire, 1989.)

As section 3.31 has shown, amygdalectomised animals clearly have a problem with distinguishing between different magnitudes of reward. None of the experiments performed so far have investigated differences as fine as two pellets versus one pellet, but in the light of previous research it is predicted that the experimental animals will show a deficit in performance. The complete predictions are that both groups of animals will find stage three significantly more difficult than stage two, but that the lesioned animals will show a greater decrease in performance.

Stage four introduces a within-session reversal to contrast with the between session reversals used previously. In the light of amygdalectomised animals' generally lower sensitivity to shifts in the magnitude of reward, it is predicted that sham animals will outperform amygdalectomised animals in this stage of the experiment.

5.1 - MATERIALS AND METHODS:

SUBJECTS:

The subjects in this study were thirty male, naive rats of the pigmented dark agouti (DA) strain (Bantin and Kingman, Hull), being approximately 5 months old at the start of the study and weighing between 200 and 250g. The animals were maintained on approximately 15g of laboratory diet per day (Beekay rat and mouse) to ensure that they did not drop below 85% of their normal body weight, with free access to water. They were caged individually, under diurnal lighting conditions (14h light, 10h dark) in a temperature-controlled room.

APPARATUS:

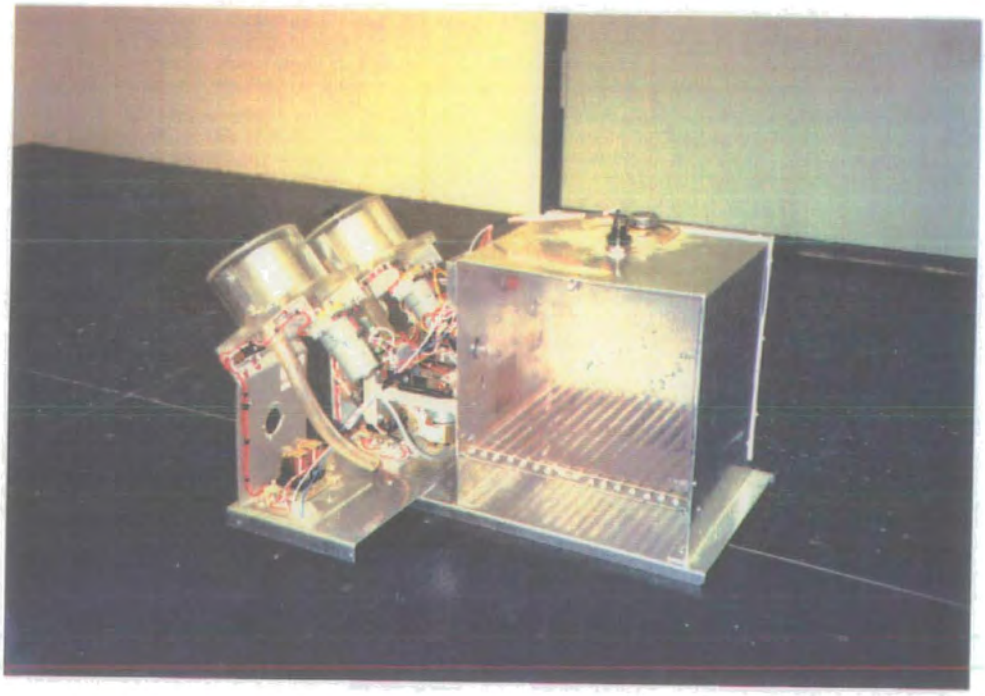
Testing was carried out in two modified operant chambers (Campden Instruments Limited, Loughborough - fig 4), under the online control of two Spider microprocessors (Paul Fray Limited, Cambridge) attached to two BBC Master microcomputers. The programs controlling the test procedure were written in Spider (a version of BASIC) by the experimenter (see appendix B). Inside the operant chambers were two retractable levers situated 7.5cm either side of a tray into which 45mg food pellets (Campden instruments) could be delivered by a dispenser. A perspex flap on the front of the food tray allowed the number of nose pokes into it to be recorded. The box was illuminated by a house light situated in the centre of the roof, and there were also lights above and embedded in each lever. A white light was located in the food tray, capable of illuminating it, and a red light was situated 8cm above the food tray.

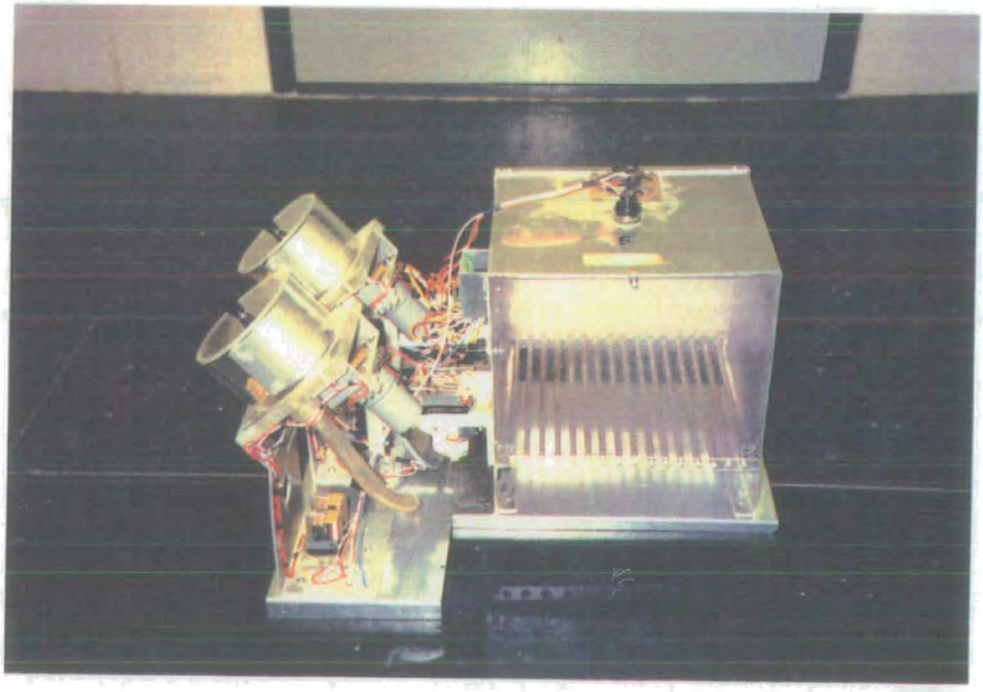
Both boxes differed from standard operant chamber design. An extra "dummy" pellet dispenser was fitted next to the original, allowing pellets to be dropped into a small tray approximately 15cm outside of the animal's chamber (to the left of the 'real' tray.) This modification was made in order to permit the experimental conditions required for stages two and three of the experiment. Photographs of the modified equipment are included in figure four.

SURGICAL AND HISTOLOGICAL PROCEDURES:

Animals were anaesthetised with an injection of 1ml/kg of a solution containing 60mg/1ml sodium pentobarbitone, administered intraperitoneally. The animal's head was then shaved, and it

Figure 4:





was placed in a stereotaxic instrument (David Kopf Instruments, Tujunga). The scalp was cut and retracted to expose the skull, and craniotomy was performed with a dental drill. In 13 animals, a total of 0.42 µl of 0.09M NMDA (Sigma chemical co., St Louis, USA) dissolved in a pH 7.2 phosphate buffer was then injected into a single site in each hemisphere using a 1 µl Hamilton syringe. A further 11 animals served as sham-operated controls. At a later date, another six surgeries were performed. Four animals received an injection of 0.5 µl of NMDA, in an attempt to increase the extent of the lesions, and two more sham surgeries were also performed. Each injection took 5 minutes, the needle then being left in place for a further 5 minutes after the injection. The stereotaxic coordinates relative to ear-bar zero (incisor-bar set +5.0 relative to the horizontal plane) were: AP +4.5, HT +1.6, LAT ± 4.1. The procedure for sham-operated controls was identical apart from the fact that the needle was lowered to HT+3.1, and withdrawn immediately. The wound was then dusted with sulphanilamide powder, and the skin was sutured. An injection of 6ml saline and 0.3ml millophylline was given to replace fluids and support respiration, and the animal was transferred to an incubator for at least one hour.

At the end of the study, the animals were killed and perfused intracardially with 5% formol saline, the brains being rapidly transferred into 5% formol saline. Subsequently, the brains were blocked, embedded in wax (Paraplast) and cut in 10 µm coronal sections. Every tenth section was mounted and stained wtih Cresyl violet, a Nissl stain..

BEHAVIOURAL PROCEDURES:

(1) Magazine training.

The rats were initially placed in the operant chambers for ten minutes, with the tray door propped open and ten reward pellets freely available in the food tray. This was intended to habituate the rats to the chamber, and to form an association between the food tray and the presence of reward. The procedure was repeated with the tray door shut, so that the animals learned to displace it to gain access to the pellets. Training stopped when the animals ate all the pellets in a period of ten minutes on three consecutive daily sessions.

(2) Autoshaping.

A randomly selected lever was introduced into the chamber, and the light above it was lit. The lever remained "out" until the animal responded by pressing it, at which point the lever was retracted,

the tray light came on, and a single pellet reward was delivered. If no response was made after 10 seconds, the lever was retracted. Autoshaping finished when an animal responded to 40 out of 48 trials over three consecutive sessions.

(3) Position discrimination.

There were three stages to the experiment. The first consisted of normal discrimination learning (as described below), the reward for a correct response being two pellets, with no reward for an incorrect response. Stage two (described below used the same basic paradigm, but with the addition of spurious secondary reinforcers. Stage three was identical to stage two, apart from the fact that the animal now received a one-pellet reward (rather than no reward) when it pressed the incorrect lever.

A session consisted of 40 trials, and the rats underwent a between session reversal when they reached a criterion of 90% correct responses in one session. The switch from stage one to stage two occurred after seven reversals, and stage three started after a further four reversals, and continued for three reversals. The experiment finished with a single session which included a reversal half way through that session.

Stage one: Each trial commenced with the white traylight and the red light above it being switched on. When the rat poked its nose into the tray, displacing the perspex door, the traylight and red light were switched off, and both levers were extended with the lights above them switched on. The rat then pressed one of the two levers, and both retracted. If the correct lever was pressed, the tray light was illuminated, and two pellets were delivered into the tray. In the case of an incorrect response, both levers were retracted with no further consequences. The next trial then commenced, the intertrial interval being around 7 seconds.

Stage two: This stage was the same as stage one, with one important addition. When the animal made an incorrect response, the tray light came on as if pellets were about to be delivered, and the dummy dispenser released two pellets into its own tray. Thus, the auditory and visual cues following an incorrect response (noise of dispenser, traylight), were almost identical to those for a correct response and the rat now had to discriminate solely on the basis of the non/appearance of the pellets in the food tray.

Stage three: In this stage, a magnitude of reward component was added. A correct response yielded two pellets; if the wrong lever was pressed, one pellet was delivered into the food tray, and a further

one by the dummy dispenser. The intention was, again, to make the result of an incorrect response sound and appear identical to a correct response. Visual cues were also matched between responses, i.e. the food tray light stayed on for the same amount of time for each.

One other aspect of stage three was distinctive. The animals were given five "free" trials, placed at random amongst the total of forty. Only the correct lever was extended for these trials. This modification arose because a potential problem with having two pellet for the S+ and one pellet for the S- is that the animal may become "satisfied" with responding on the incorrect lever and receiving just one pellet per trial. The presence of the "free" trials forced each animal to press the S+ lever, so guaranteeing experience of both magnitudes of reward. The criterion remained at 90%, but the "free" trials gave the animals an additional score of 12.5%. This (effectively) lower criterion proved appropriate, as most animals found stage three difficult. The six animals which were operated upon in the second batch of surgeries did not complete stage three due to time constraints.

Stage four: The experiment terminated with a single session (of 40 trials) similar to those in stage three, which included a reversal exactly half way through it, and omitted the "free" trials.

5.2 - RESULTS

5.21 - HISTOLOGY:

The aim of the lesions was to damage all nuclei, but in general the lesions were more variable than intended. They are represented graphically in fig. 5, summarised at four rostrocaudal levels. The lesions were centered around the basal and accessory basal nuclei[1]. Both of these nuclei consistently sustained heavy damage, and the lesions frequently extended into the more dorsal parts of the medial nucleus and the lateral parts of the anterior cortical nucleus. The central nucleus suffered more intermittent subtotal damage, and the lateral nucleus was always at least 50% spared. One disappointing aspect of the lesions was their asymmetry. Four animals were eliminated from the following analyses, due to either very asymmetrical or very small lesions (this left a total of 13 lesioned and 13 control animals). In general, the right hemisphere lesions were situated more rostrally than the left. The neurotoxin appeared to spare fibres of passage and vacuoles developed in only two animals. They were unilateral and rostrocaudally circumscribed.

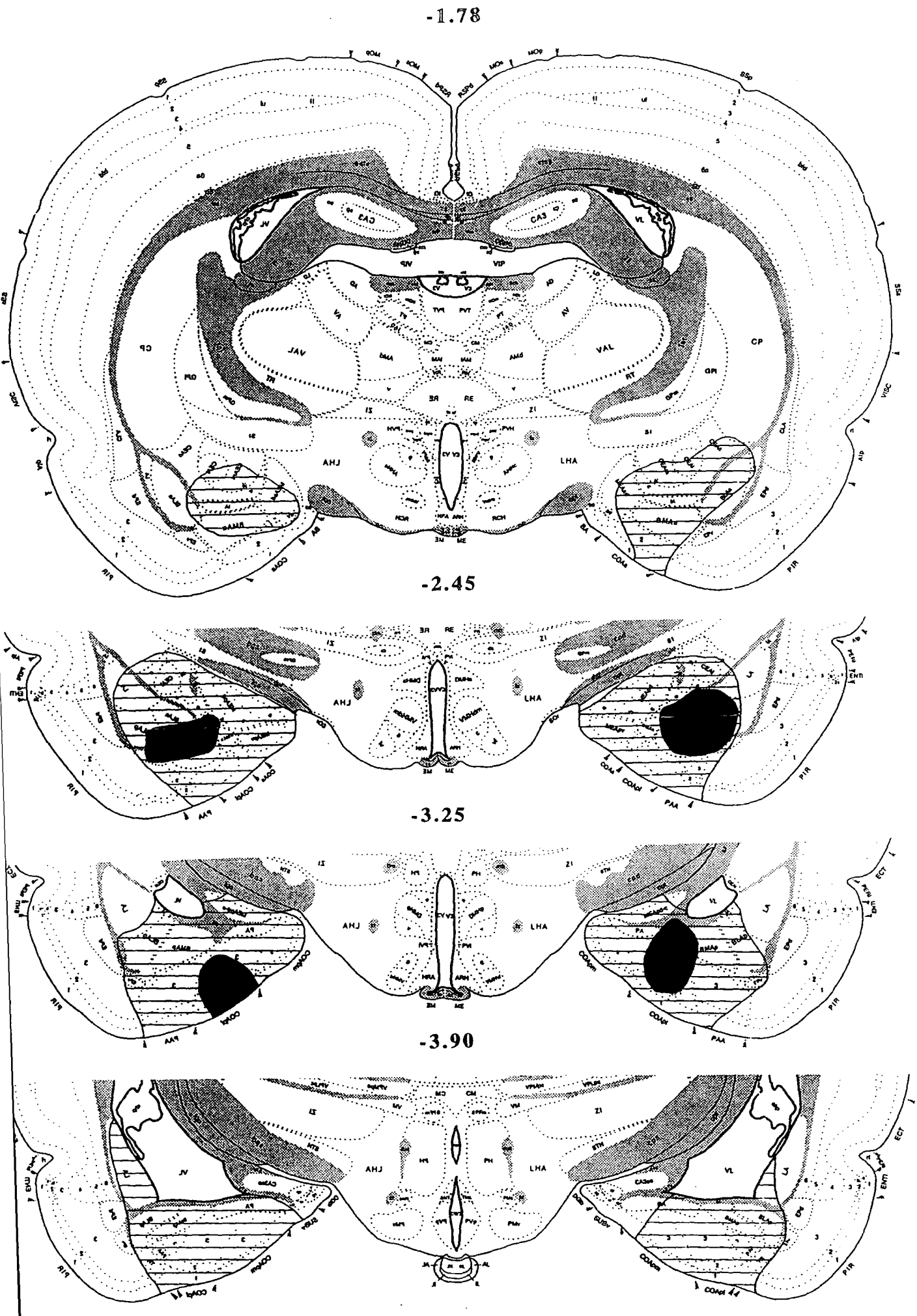
[1] "basolateral" and "basomedial" respectively

Extraamygdaloid damage was minimal. Slight invasion into the most ventral parts of the putamen was observed in only two animals. The lesions did not extend beyond the rostrocaudal limits of the amygdala.

Figure five: The hatched areas represent the extent of the largest lesion at that rostrocaudal level, the filled-in black areas the smallest. The diagams are taken from Swanson (1992).

Figure 5:

Antero-posterior level relative to bregma:



STAGES ONE, TWO AND THREE:

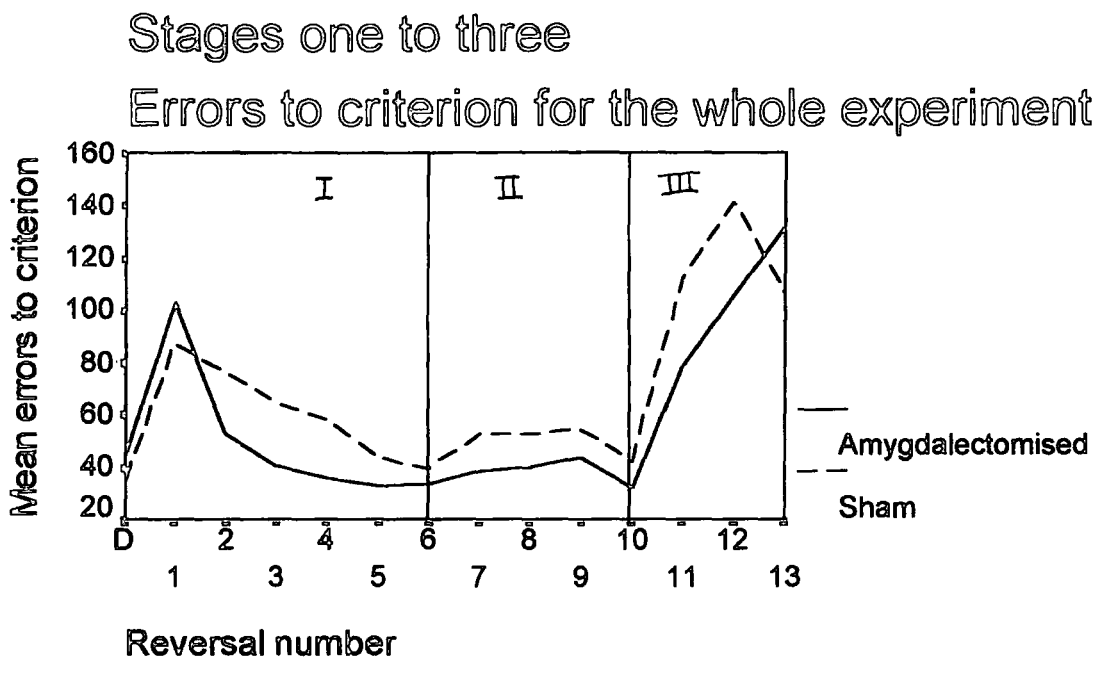


Figure 6.1: This is provided to give an idea of how performance (measured by errors to criterion) varies over the course of the whole experiment, enabling comparison of stages one, two and three. "D" here represents the number of errors to first criterion (a basic discrimination, not a reversal.)

Stage one consisted of reversals one to six, stage two of seven to ten, stage three of eleven to thirteen.

5.22 - STAGE ONE:

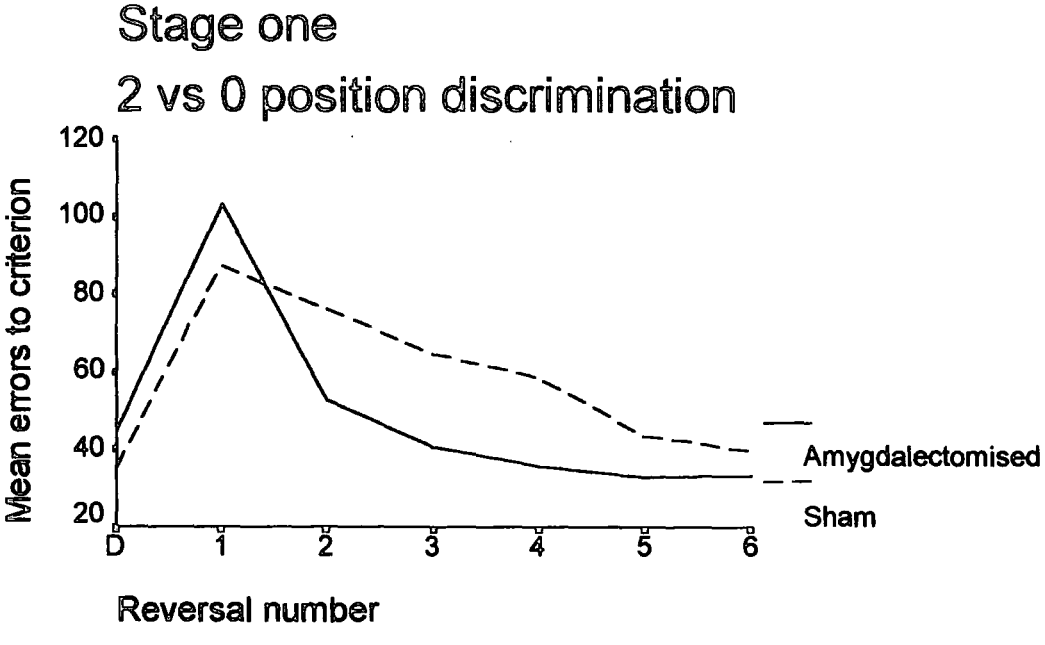


Figure 6.2: Average errors to criterion for both groups for the whole of stage one.

A t-test of errors to criterion on the initial discrimination shows that the two groups do not differ significantly in performance ($t,24=-0.88$ $p=0.389$). An ANOVA was performed for the reversals one to six, using the between subjects factor "lesion" and within subjects factor "reversal". Unsurprisingly, there is a strong effect of reversal, ($F\ 5,120=14.03$, $p<0.001$), but no effect of lesion status or interaction between lesion and reversal was seen. Stage one was also analysed in two parts, in order to attempt to replicate Kentridge et al.'s (1991) finding of differential results after a higher number of reversals. Reversals one to three were the first part, and reversals four to six were the second. The first three reversals show a sharp fall in mean errors to criterion. A two-way analysis of variance with between subjects factor "lesion" and within subjects factor "reversal" (1-3) reveals that there is no lesion effect here, but the effect of reversal is highly significant at ($F2, 48=9.12$ $p<0.001$). The change in scores between reversals one and two hints at the presence of an interaction between lesion and reversal, but the ANOVA shows that this does not reach significance ($F\ 2, 48=2.46$ $p=0.096$).

In the light of Kentridge et al's (1991) finding of a greater lesion effect with later reversals, an ANOVA similar to that described above (within: reversal (4-6), between: lesion) was performed. This shows that the amygdalectomised animals are making significantly fewer errors before reaching criterion than the shams ($F1, 24=6.75$ $p=0.016$). The effect of reversal is no longer significant ($F2.48=2.36$ $p=0.105$), and there is no significant interaction. It is worth noting that at reversal six, there is no longer a significant difference in errors to criterion between lesioned and sham groups ($t\ 24=1.32$ $p=0.199$).

In order to fathom what may be going on immediately after a reversal - for example, how quickly a rat starts exploring and responding on the new S+ lever - an analysis of the first forty trials post-reversal has been performed for the second part of stage one (reversals 4-6). Values were obtained as follows. For each rat, on each of the first forty post-reversal trials, it was ascertained whether or not this rat had responded correctly or not after reversals three to five (N.B.: *after* reversals three to five corresponds to the errors to criterion scores for reversals four to six, where the significant effect of lesion was found). For example, taking rat five, did he respond correctly on the sixteenth post-reversal trial after reversals three, four or five? If he responded correctly after reversal three, but not after reversals four or five, he would be assigned a score of 33% - one out of three trials correct.

Averages were obtained for both experimental groups for all forty trials, and the results are displayed below in figure 6.3.

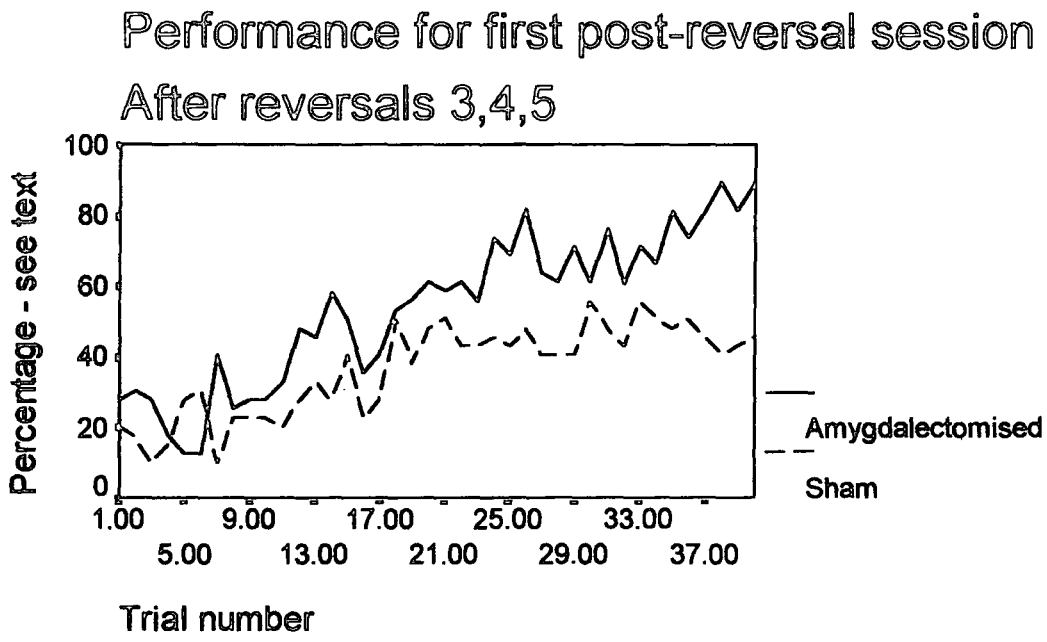


Fig 6.3: Percentages correct (see above) for the first sessions after reversals three, four and five

In order to permit statistical analysis of these scores, they were averaged by animal for trials 1-10, 11-20, 21-30 and 31-40 (see fig 6.4) and an ANOVA was performed on these summary scores. The analysis of variance discerned three significant effects - of lesion, reversal and of an interaction between the two (test statistics $F_{1,24}=24.08$ $p<0.001$, $F_{3,72}=61.45$ $p<0.001$ and $F_{3,72}=5.62$ $p=0.002$ respectively). Scores were higher for amygdalectomised animals and there was an overall elevation in scores with further trials. The interaction was investigated with a post-hoc Newman-Keuls test. In the summaries of trials 11-20, 21-30, 31-40, the lesioned animals scored significantly higher than the shams; for trials 1-10, this tendency is also present (amygdalectomised - 24.84%, sham - 20.47%) but nonsignificant.

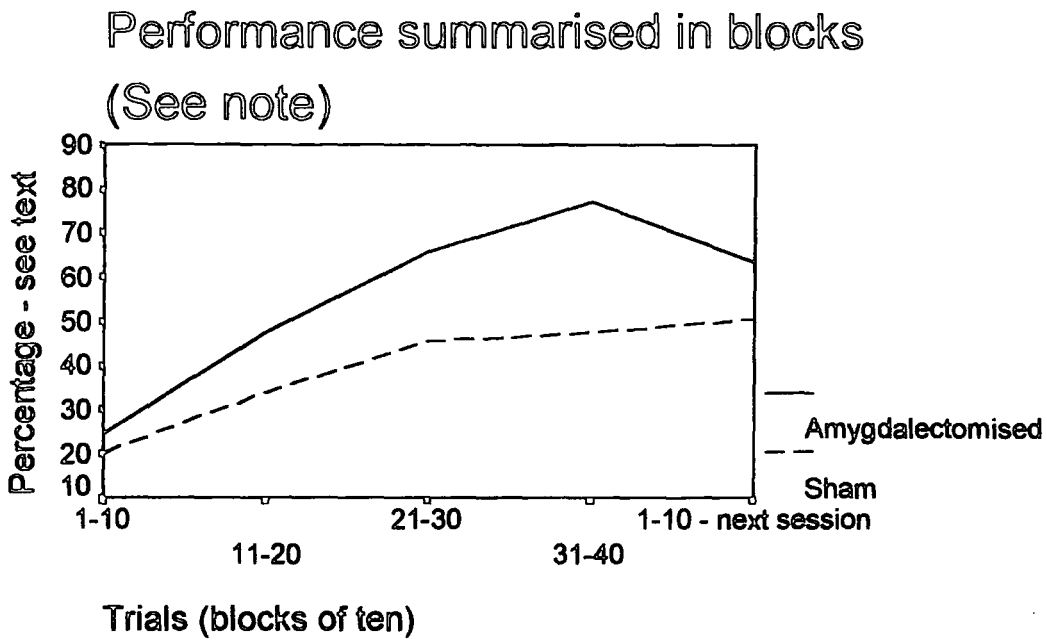


Fig 6.4: This graph shows the data summarised in blocks of ten. All forty trials of the post-reversal sessions are represented here, as well as the first ten trials of the session after that.

Fig 6.4 summarises fig 6.3 in blocks of ten, and also adds similar data for the first ten trials of the session following the postreversal session. This was included in order to study the change in scores between testing sessions (e.g. between days). An ANOVA was performed on the summaries of the last ten trials of the first session and the first ten trials of the next, and this shows a clear interaction between day of testing and lesion ($F_{1,24}=7.91$, $p=0.01$); the scores of the amygdalectomised animals drop significantly more "overnight" (between sessions) than those of the shams. The ANOVA also shows an effect of lesion, the scores of the lesioned animals remaining overall higher than the shams ($F_{1,24}=21.94$, $p<0.001$).

5.23 - STAGE TWO:

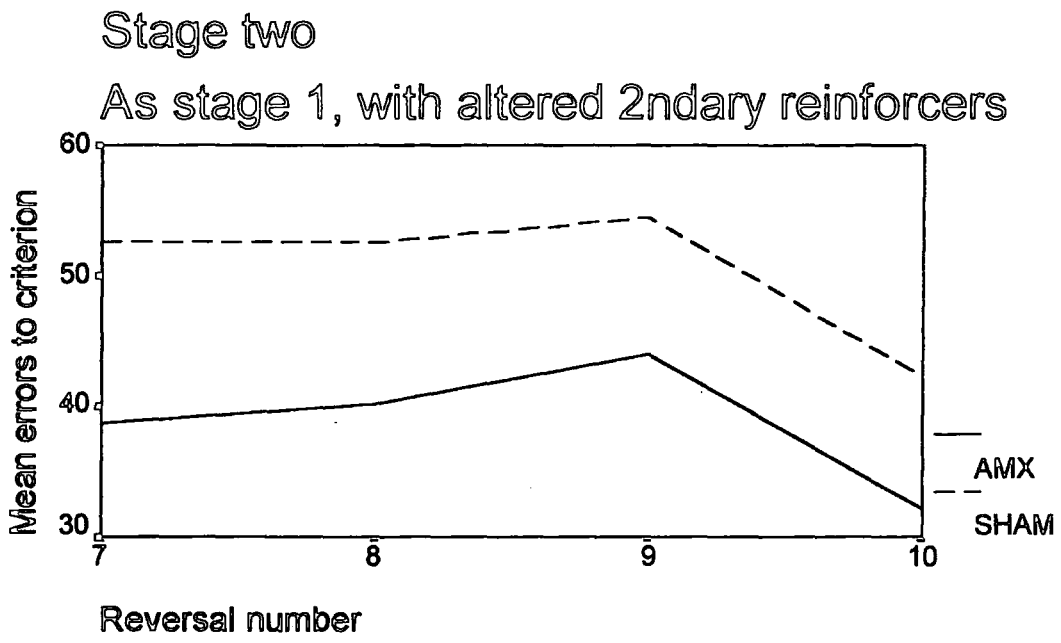


Figure 6.5: Average errors to criterion for both groups for the whole of stage two.

Looking at reversals seven to ten, two effects stand out; amygdalectomised animals are still making fewer errors before reaching criterion than shams, and there's a sharp drop in errors between reversals nine and ten for both groups. A two way ANOVA (lesion x reversal) confirms this - there are significant effects of lesion ($F\ 1, 24=5.40\ p=0.029$) and of reversal ($F\ 3, 72=3.17\ p=0.029$), but no trace of an interaction ($p>0.95$).

The analysis is limited however if it only includes reversals seven to ten. For the purposes of this experiment, the change in errors to criterion between reversals six and seven is particularly interesting (this highlights the animals' initial response to the change in experimental conditions - the equalising of secondary reinforcers). An ANOVA on reversals six and seven with the within-subjects factor of reversal shows no interaction between lesion and reversal ($f\ 1, 24=0.82, p=0.373$) - this means that the increases in scores associated with the introduction of stage two do not differ by lesion.

5.24 - STAGE THREE:

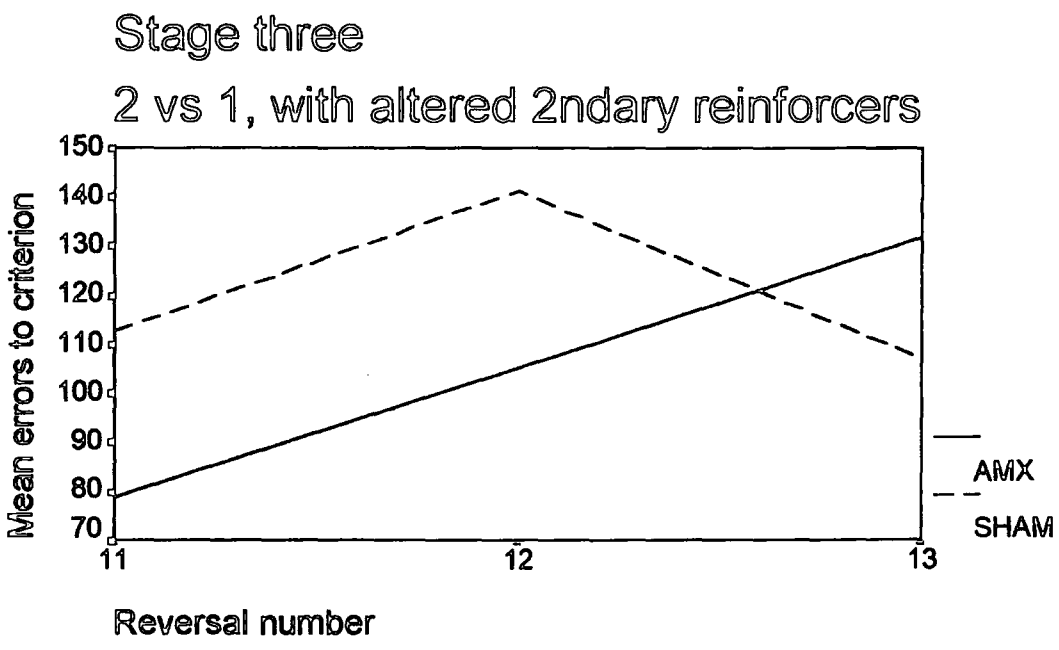


Figure 6.6: Average errors to criterion for both groups for all of stage three.

At reversal eleven there is a dramatic increase in the number of errors to criterion for both groups. This corresponds to the introduction of the 2 vs 1 pellet discrimination task. The initial increase at reversal eleven is slightly less for amygdalectomised animals than for shams, but the an intriguing aspect of stage three is that the errors of the lesioned animals keep rising, whereas they tail off for the shams at reversal thirteen. Whilst this effect does not reach significance, it is entertaining to speculate what would have happened at reversal "14", had it existed. A two-way ANOVA ('reversal' vs 'lesion') shows no significant effects of reversal or lesion. The variance of the data in stage three was huge, however - for example: errors to criterion at reversal 12 - mean=125, standard deviation=87.34, range 2-357 - and this may in part explain the lack of an effect of reversals. Also, the six animals from the second batch of surgeries did not complete this part of the experiment, and are excluded from the analysis[2]. No interaction was present ($F_{2, 34}=1.75$ $p=0.189$), despite the suggestive crossover at reversals twelve and thirteen. To investigate the initial deflection of scores due to the introduction of the 2 vs 1 task, an ANOVA was performed on reversals ten and eleven - no interaction was found between lesion and reversal ($F_{1,17}=0.16$, $p=0.696$), so the amount of increase in scores upon the introduction of reversal three did not differ between experimental groups.

[2]final n for stage 3: amygdalectomised=9, sham=11.

5.25 - STAGE FOUR:

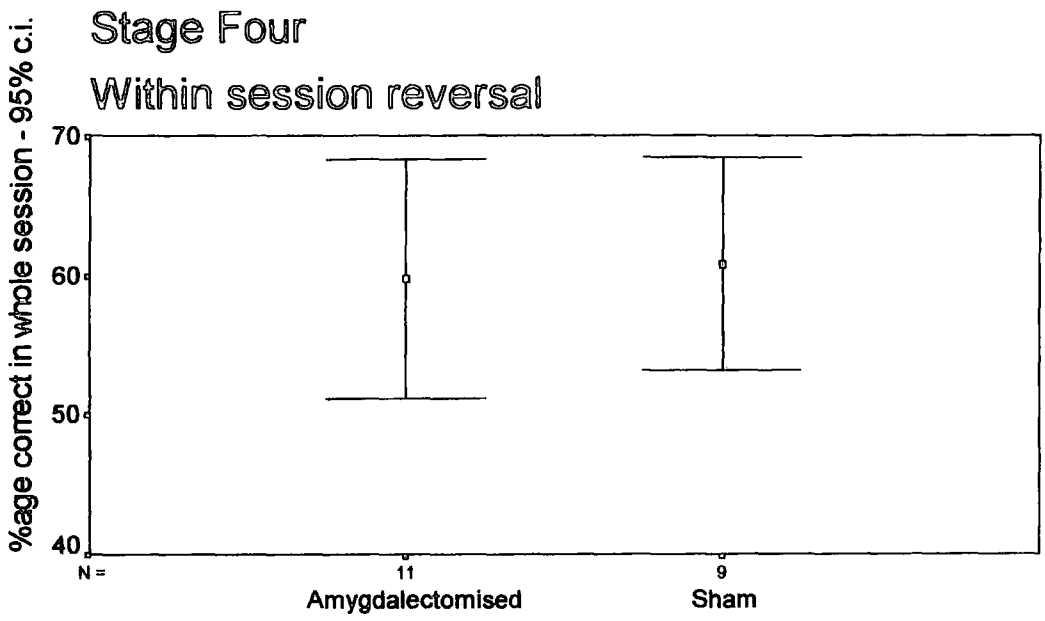


Figure 6.7: this graph shows the average percentage correct for both groups across the whole session.

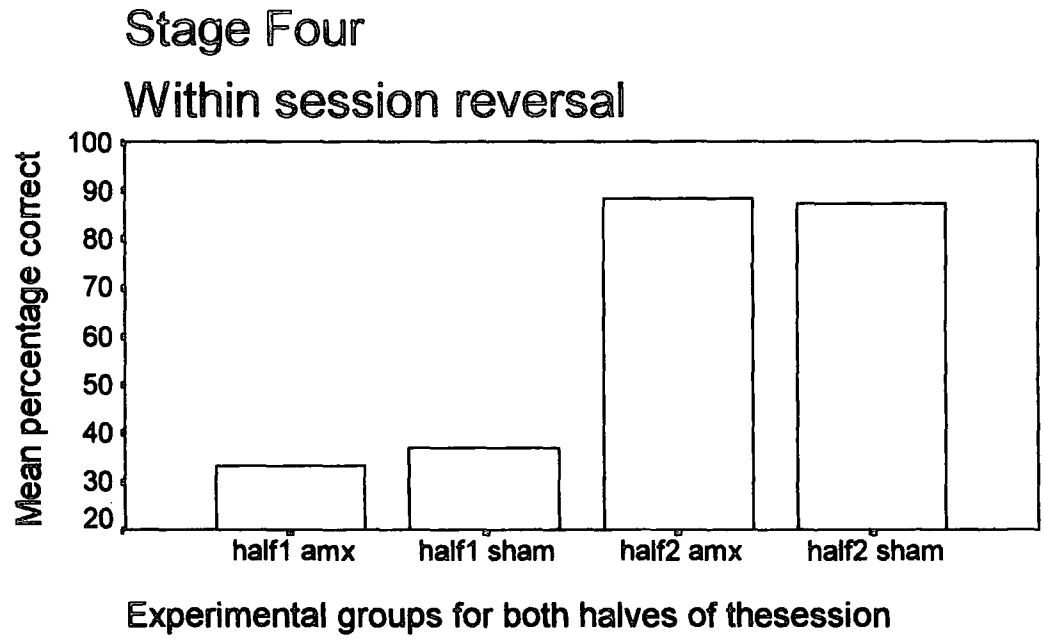


Figure 6.8: this graph displays the percentage correct scores for the first and second half of the final session, highlighting the increase in correct reponses.

For stage four, a percentage correct score was obtained for the whole session, and also for the trials before (half1) and after (half 2) the reversal. These are depicted in figs 6.7 and 6.8. An ANOVA of the data contained in fig 6.8 reveals no effect of lesion or interaction ($p>0.5$), but as is evident from

the graphs, there is a highly significant increase in scores in the second half of the session ($F_{1,18}=119.55$, $p<0.001$) compared to the first.

5.3 - DISCUSSION:

5.31 - Stage one:

The initial discrimination and first two reversals of stage one follow an anticipated pattern. A certain number of animals will start on the correct lever by chance, explaining the relatively low errors to criterion for the initial discrimination. (It is significant in the light of the meta-analysis, however, that the two groups do not differ in performance on the initial discrimination.) Reversal one is then the first "genuine" switch that the Ss have to learn, and errors to criterion are correspondingly high. Animals clearly begin to learn the rules involved during and after reversal one, as they reach criterion at reversal two substantially more quickly, and errors to criterion continue to drop throughout the rest of stage one, flattening out at reversals five and six. Kentridge et al. (1991) note that their animals display a "learning set" - they show continued increments in performance in reversals four to seven of their experiment - but they rule out the possibility that failing to use a learning set was the basis of the lesion effect that they observed. The shams in reversals two to six show a marginal (nonsignificant) facilitation of learning with these reversals, but the lesioned animals do not. This might suggest the absence of a learning set in amygdalectomised animals, but is in fact more likely to be due to these rats having reached a "ceiling" in performance. By reversal four, they are reversing about as fast as such animals can, and so they show no further improvement over reversals five and six. This means that no firm conclusion can be reached as to the presence of a deficit in learning set formation.

The fact that amygdalectomised animals reverse faster than shams from reversals four to six requires careful analysis and explanation. It is a counterintuitive result in terms of past experiments (see section 4) and also in terms of the simple (but not entirely unfounded) prejudice that brain lesions should not produce improvements in performance on cognitive tasks. In order to explain these results, it is necessary to try and rediscover what resources are required for an animal to successfully perform a reversal, and what might make one animal reverse faster than another, or, indeed, more slowly.

Two hypotheses have commonly been advanced to account for the cognitive mechanisms behind reversal deficits. If an animal were to show a strong tendency to persevere in its responses, it would be much slower than controls to switch to the new S+ lever after a reversal. (For a recent

example of a brain lesion producing a perseverative reversal deficit, see Ridley et al. 1993.) Alternatively, it is also difficult to see how an animal could effectively and rapidly perform reversals if it was impaired in its ability to link discriminanda with their reward values. Either of these tendencies, if reversed, could be used to explain superior reversal performance, but not without problems; if the lesioned animals persevered less, they might switch to a new S+ lever more quickly, but would surely encounter problems in staying on that lever until a criterion of 90% was reached. (Perseveration is defined as the tendency to persist with a response after it has ceased to be adaptive. It is hard to see how any reading of the opposite of this tendency would be beneficial in staying on a lever until a 90% criterion was reached.) Similarly, it is easy to conceive of an animal having problems with forming stimulus-reward connections, but harder to envisage an animal *superior* at creating them. An agnosic patient seems more comprehensible than a "*hyper*gnosic" patient who was greatly superior to undamaged people at object recognition, and the hypergnosic patient would seem to underline the fact that we only have an unclear idea of how object recognition occurs at all. A similar situation appears to occur here when looking at the question of "superior" stimulus-reward association abilities, and so it is necessary to return to the data for clues that might explain the lesioned animals' superior reversal performance.

Jones and Mishkin (1972) note that animals generally go through three stages of response after a reversal. Initially, they persevere on the incorrect lever, then they appear to press on either lever at random, before finally showing a bias towards the correct lever. This sequence emphasises the fact that the animal has to both "unlearn" to respond on the old lever, and then make the new connection between the other lever and reward. The data depicted in figs 6.3 and 6.4 allow us to examine the unfolding of these three stages across the first post-reversal sessions. To provide an anchor point for the analysis, we can assume that a score of around 50% corresponds to the rats being at Jones and Mishkin's (1972) "random" stage. With this in mind, it becomes clear that the sham group never get further than this stage in these post-reversal sessions. For trials 31-40, their average score is 47.68%, whereas the amygdalectomised animals have reached an average of 70.32%, which is highly suggestive of a clear move to the new S+ lever. The amygdalectomised animals also appear to persevere on the S- lever for less time than the shams. The shams appear to be clearly persevering for

the first 20 trials, whereas the scores of the amygdalectomised rats in trials 11-20 suggest that they are reaching the "random" stage already. In trials 21-40, the control animals appear to be at the "random" stage, whereas the lesioned animals pass rapidly from this stage to the acquisition of a response to the new S+. It seems that not only do the amygdalectomised animals persevere less, but they also acquire a response on the new S+ more rapidly than shams.

The need for an animal to "unlearn" a response to the old S+ after a reversal has already been referred to. It is plausible that amygdalectomised animals forget their stimulus-reward associations more quickly between sessions, thereby facilitating their move to the new S+ and enhancing their performance. Two predictions arise from this idea; first, that the performance of the lesioned animals will be greater in the first ten trials after reversal, secondly that the amygdalectomised animals will show a greater drop in performance between the end of the first post-reversal session, and the beginning of the subsequent session due to the forgetting of S+.

The results show the first prediction to be incorrect, and the second equivocal. Post-hoc tests reveal no significant difference between the lesioned and sham groups in the first block of ten trials after a reversal. The lesioned animals, however, clearly do display a greater drop in performance compared to shams between the first and second post-reversal sessions. This may appear to support the "faster forgetting" hypothesis, but in fact no such strong conclusion can be drawn. At the end of the first session (trials 31-40), lesioned animals scored 77.4%, shams 47.7%. The next day they scored 63.5% and 50.7% respectively. Using Jones and Mishkin's (1972) schema, the shams are responding randomly (near 50%) in both sessions, whereas the lesioned animals have clearly switched to the S+ at the end of the first session, and have to an extent forgotten this association by the next day. There is no drop in sham performance because there is no association with the S+ yet formed for them to forget. There are not sufficient grounds from these results to conclude that the amygdalectomised animals forget discriminanda-reward associations faster than shams.

Other possible causes of superior reversal performance must be considered. Relevant evidence is possibly forthcoming from previous data on neophobic behaviour in amygdalectomised rats, however; it could be argued that reduced neophobia or increased exploration would dispose an animal to take advantage of the correct lever more quickly. Previous experiments (see sections 2.5,

3.24) have broadly indicated that amygdalectomised rats tend to explore more and are less neophobic, and this lends face validity to the idea. The nature of the experimental paradigm makes this unlikely, however. By the time the animals reach reversal four (when the lesioned animals begin to reverse more quickly) the fastest of them had already made over 400 responses distributed equally over both levers, and had spent about 4 hours in the operant chambers - not including magazine training and autoshaping. In this context, it seems unlikely that changes in neophobia or exploratory behavior played a significant role.

One last factor that could effect reversal performance is the motivational status of the animals. For example, hungrier animals might be expected to reverse more quickly, and given the substantial dietary/eating behaviour changes seen in Klüver-Bucy syndrome, this is a possibility in amygdalectomised rats. Lorenzini et al. (1991) report no overall quantitative change in feeding , but a shift towards eating in the 'light' period in lesioned subjects - this lends face validity to the idea. Hunger alone seems unlikely to be the cause of the elevation in reversal performance, however. After a reversal, the behaviour of the more hungry rat will depend entirely on its cognitive status; if it still associates the (now) S- lever with reward, it will press it with great enthusiasm, or if this association has begun to extinguish, it will explore the other lever more readily. Hunger or motivational status appear to be a secondary variable to the cognitive processes involved.

In the absence of other plausible explanations, it seems that the lesioned animals in this experiment forged new stimulus-reward associations and "forgot" old ones more quickly than shams. It is salutary to remember, as Mackintosh (1974) puts it, that there are "difficulties for any simple, conditioning-extinction theory of discrimination learning", due to such findings as learning sets and the overtraining reversal effect. There appears to be something extra, above and beyond the formation of new stimulus-reward connections with each reversal in highly trained animals. It is perhaps to this factor (although most accounts of it are "unsatisfactory and vague" - Mackintosh) that we must turn to to explain the results, though it remains difficult to see how a lesion could improve this factor, whatever it may be. This result remains entirely unanticipated, however, and it raises the question of whether the animals (in either group) managed to perform the task in some fashion that did not involve the formation of position/lever-reward associations.

The design of the operant chambers is asymmetric, as one "wall" is a transparent perspex door through which the chamber itself is accessed. This could bias the animals towards one lever, but in a task involving so many reversals, this is not likely to have a great effect. Also, at the beginning of the experiment, the "start lever" (left/right) of each animal was chosen randomly. After a reversal, the animals' behaviour appears to conform to the phases outlined by Jones and Mishkin, which also implies that position/lever-reward associations are governing their behaviour. It is extremely hard to imagine any strategy or spurious environmental cue that could consistently ensure that rats reverse faster and then stay on the correct lever more consistently until they reach criterion.

5.32 - Stage two:

The same questions about the nature of reversal learning haunt stage two, but in this case are overshadowed by the implications of the added manipulation of secondary reinforcers. This alteration to the experimental paradigm caused a general elevation in the difficulty of the task, as noted in the results section. Given that the sham data shows that manipulation appeared to "work", this variable should provode a direct test of the theories of Gaffan (1992) and Everitt & Robbins (1992). The elevation in task difficulty clearly shows that both groups were using the secondary reinforcers as cues before their alteration.

A differential elevation in errors between groups due to the introduction of stage two would decide the issue - for example, if lesioned animals displayed a greater elevation in errors for reversal eight, this would provide clear support for Gaffan's account of amygdala function. Unfortunately, no such result is forthcoming - an ANOVA of reversals six and seven shows no interaction between lesion and reversal ($f_{1,24}=0.82$, $p=0.373$). Such an interaction across all of stage two would be equally revealing, but this is also almost prominently absent ($p>0.95$). The fact that there is a significant reduction of performance at the beginning of stage two, however, shows that both groups *were* using the secondary reinforcers as salient cues in performing the discrimination.

Apart from the interaction, Gaffan's theory would predict a generally higher number of errors to criterion amongst lesioned animals compared to shams, and Everitt & Robbins the opposite. Here, the data appear to support Everitt & Robbins' theory - but the more rapid reversal of the lesioned animals could be interpreted as a continuation of this tendency from stage one.

Given these results, Gaffan's theory is clearly unsupported, but emerges relatively unscathed, as it pertains quite specifically to certain object discrimination tasks in the monkey. The faster reversals of the lesioned animals could be seen as a confirmation of Everitt & Robbins' theory, but as this phenomenon was also present in stage one, this is not conclusive. Also, the more convincing evidence - a differential increase in task difficulty on the introduction of stage two, or an interaction - is absent.

5.33 - Stage three:

A 2 vs 1 pellet discrimination is clearly a difficult task for a rat, as the introduction of this stage elevates errors to criterion to a level above that seen when the animals learned the initial discrimination task - despite the 12.5% "free" score. It is noted in the results section that there is a great deal of variance in the data, but it is equally important that all of the animals *did* complete three reversals in this stage. Given that the animals could successfully perform the task, it is all the more striking that no lesion effect was found between groups. The past literature on the response of amygdalectomised animals to magnitude of reward changes is quite unequivocal - lesioned animals do not behave in a way that shows them to be as sensitive to such changes as intact animals, across a wide range of tasks. However messy the data, the equality of performance between the experimental groups must be explained.

None of the past literature has used a distinction as fine as two pellets vs one to assess the function of amygdalectomised animals. It seems reasonable to assume that whatever problems were revealed by earlier experiments should be accentuated by the introduction of a finer discrimination. So either these animals did not exhibit the same difficulties as those in other studies, or perhaps the task utilised in stages three and four was tapping a somewhat different ability.

The data appear to allow one possible alternative to this conclusion. Throughout the second half of stage one and all of stage two, there is a significant effect of lesion - the amygdalectomised animals reversing faster than the shams. The lack of a lesion effect in stage three signifies the disppearance of this difference - possibly indicating the presence of a subtle magnitude of reward deficit in the amygdalectomised animals. The most straightforward way to test this idea was already used in stage two; an ANOVA to test for differential changes in scores when stage three is introduced.

If the above idea is true, then the amygdalectomised animals should show a greater increase in errors to criterion between reversals ten and eleven than shams. As the results show, this is not the case ($f_{1,17}=0.16$, $p=0.696$).

As section 3.31 tries to emphasise, it is not at all clear what a "magnitude of reward problem" is, as the deficit has generally only been described in functional terms rather than by reference to processes that might underpin it. Salinas et al. (1994) have made one attempt in this direction, suggesting that amygdala inactivation eliminates the site at which memories for a downward (aversive) shift in reward might be stored. It seems to stretch their theory to claim that a deficit seen in stage three might be due to an animal forgetting about the aversive consequences of the drop from 2 pellets to 1 as it continues to respond on what is now the wrong lever. If this is a prediction from their theory, it has not been supported. Indeed, it appears that the animals involved in this experiment display no "magnitude of reward problems" as previously defined.

5.34 - Stage four.

The conclusions above are echoed by the results of stage four. As there is no difference in performance between groups on either half of the final session (in fact, the scores are exceptionally similar), there are clearly no grounds for postulating a magnitude of reward deficit here. There are grounds for questioning the methodology of stage four, however; the lack of any significant differences in scores here is perhaps due to the poor calibration of the task. The animals were observed to respond principally on the incorrect lever for the first half of the session (persevering from the previous session), and to persist on this lever for the second half (when it was again the "correct" lever). The results appear to confirm this interpretation - low scores for half one, high for half two. It was hoped that the animals' response to the first (beginning of session) and second (within session) reversal would allow in-depth observation of their responses to two rapid changes in reward contingency. This might have worked with a 2 vs 0 pellet discrimination, or perhaps with a very long session (e.g. 40 trials before and after the reversal), but the difficulty of the 2 vs 1 task appears to have led the animals to treat half 1 as a temporary drop in reward between the previous session and half 2.

6 - EXPERIMENT TWO: RESISTANCE TO CAPTURE.

This experiment was designed to test a hypothesis which was formed when handling the amygdalectomised animals. From Weiskrantz (1956) onwards, it has generally been agreed that amygdalectomised animals show attenuated fear/aversive reactions to handling, and to cues associated with handling. This contrasted sharply with day-to-day observation of the lesioned animals in the currect study, which appeared to be dramatically more averse to handling than shams, showing more tendencies to escape, claw and bite. To test this observation formally, the methodology used follows Seggie (1971), using independent "blind" raters to assess the resistance of each animal to capture. A 1 to 7 scale replaces Seggie's 0 to 4 scale, and it also does not include specific descriptors of behaviours for each point on the scale, relying rather on the experience of the raters. The ratings were made two months after surgery to ensure that the change in behaviour was not transient.

6.1 - MATERIALS AND METHODS:

SUBJECTS:

The same subjects were used in this experiment as in experiment 1.

APPARATUS:

The ratings for this section of the experiment were made by two technicians who had considerable experience in handling rats. They had handled these animals previously, whilst their lesion status was clearly displayed on their cages, making the "blind" rating and random handling order particularly important. They recorded their ratings on a sheet which contained the number of each of the rats in a pseudorandom sequence, and a scale of 1 to 7 next to each number. The instructions on the sheet were as follows: "Please handle the animals in the given order, and rate them from 1 (very docile) to 7 (very aggressive), 4 being about average. Thankyou."

PROCEDURE:

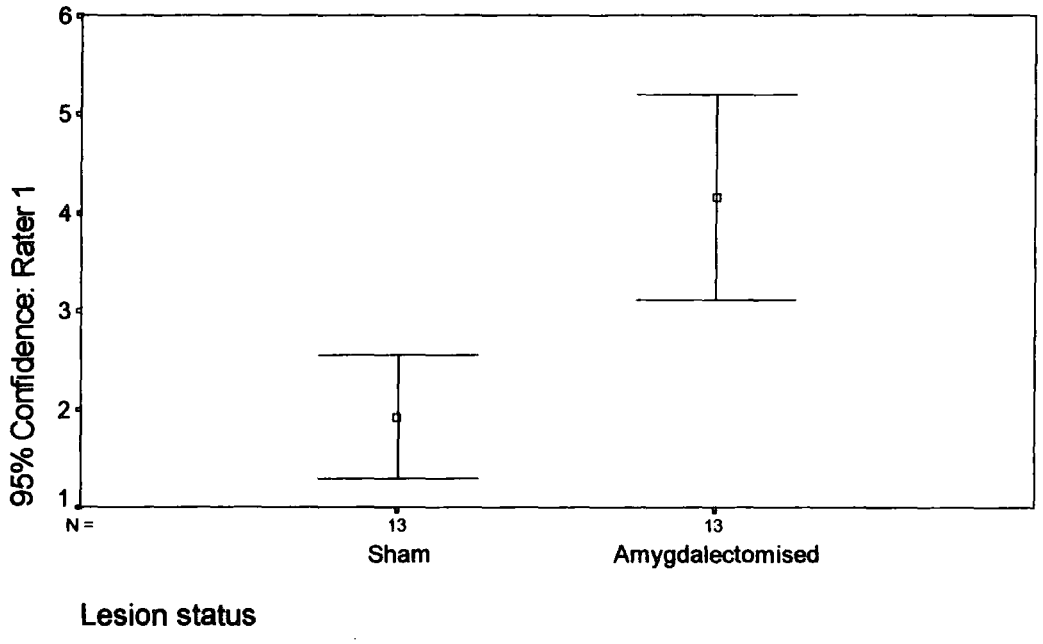
The ratings were made two months after surgery, whilst the animals were still engaged in daily testing on experiment one. Either in the course of routine cage-cleaning or separately, the raters handled the Ss for 1-2 minutes, and rated them for aggressivity/resistance to capture. The lesion status of the Ss

was not known to the raters, and the Ss were handled in a pseudorandom order. On the same day, the Ss were weighed to ensure that the raters could not have differentiated their lesion status by weight.

6.2 - RESULTS:

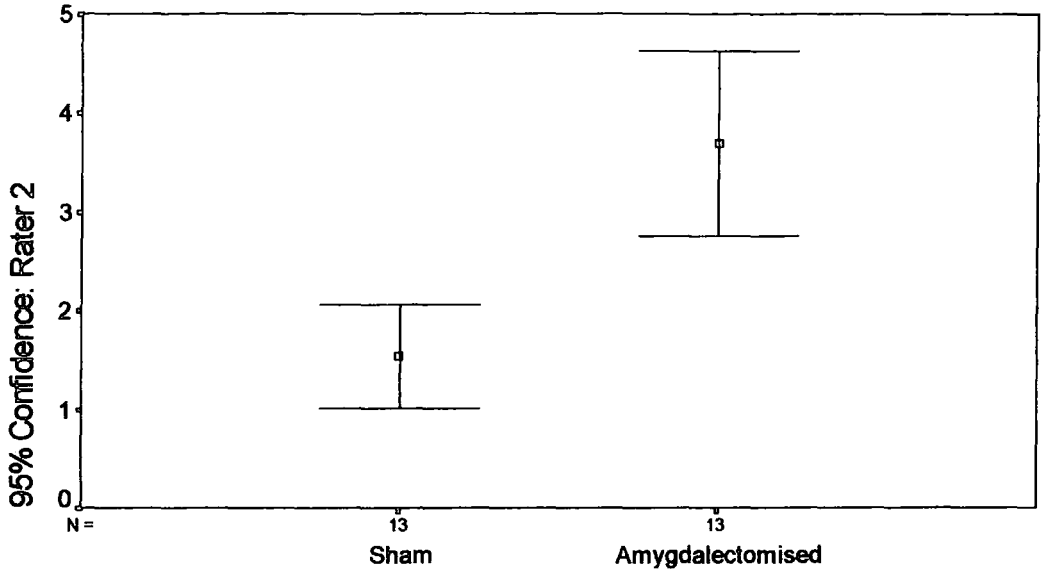
Both raters found the amygdalectomised animals to be significantly more resistant to capture than shams: rater 1, $t(28)=4.1$ $p<0.001$; rater 2, $t(27.09)^1=5.18$ $p<0.001$. The judgements of the two raters were significantly correlated at $r=0.68$ ($p<0.001$). It is highly unlikely that the raters were able to tell which rats were lesioned by weight, as neither group was significantly lighter - $t(28)=0.52$, $p=0.61$. The following error-bar charts show the mean rating for the two raters with their associated 95% confidence intervals.

Rater One:



[1]adjusted for unequal variances between groups.

Rater two:



A final calculation was made to check Aggleton and Passingham's (1982) suggestion that there is a relationship between reversal performance and hypoemotionality. The ratings obtained here were correlated with the sum of errors to criterion for each animal for reversals 1-6 (all of stage one after the initial discrimination). No significant association was found for either rater (both p's >0.1, r from -0.26 to -0.32).

6.3 - DISCUSSION

The results of experiment 2 confirm the informal predictions that resulted from handling the lesioned animals. As this goes against the grain of most previous research, it requires careful explanation.

A small, and mainly uncited tradition exists in the past literature, however. Both Aggleton and Passingham's (1982) and Rosvold et al.'s (1954) cohorts of amygdalectomised monkeys contained individuals that became *more* aggressive after surgery. More compelling than this, however, are Robinson's (1963) results which show that amygdalectomised rats were excessively fearful as a group, rather than in individual cases. The most recent results that concur with this tradition are those of Cahill and McGaugh (1989). Citing their 1989 findings, Cahill and McGaugh (1990) note that "the NMDA AC [amygdaloid complex] lesion that has been used in this laboratory has consistently produced rats that are hyperreactive to handling". These findings are highly significant, as there is no question of them being

caused by extraamygdaloid cortical damage (possible in Rosvold et al) or by damage to fibres of passage (possible in Robinson's study).

Recent results from Adamec and Morgan's (1994) study may offer an explanation for these contradictory findings. Localisation appears to have been of great importance in this study, as it was found that kindling anterior amygdaloid foci increased anxiety, whereas posterior electrode sites decreased it (anxiety was measured with an elevated plus-maze and a hole board test). It becomes clear that manipulating different parts of the amygdala can cause entirely opposite emotional responses.

Awareness of Adamec and Morgan's results, and also of the small but insistent "hyperemotional" literature decreases surprise at the results of experiment 2. Put together, all of these findings indicate that there is an extra level of complexity in the amygdala's role in emotional behaviour, and it also appears to refute the idea of the "hypoemotional" deficit being solely due to a disruption of visual stimulus-reward connections (though this is doubtless the case at times - Downer, 1961).

One final possibility remains as a potential cause of the hyperreactive behaviour seen. An experiment was performed by Pinel, Treit and Rovner in 1977 in which amygdala-kindled animals were shown to be much more reactive to handling than shams. Ermakova et al (1989) induced epileptogenic damage using infusions of 0.2% kainic acid into the amygdala, and it is possible that the NMDA lesions used here were having a similar effect, and the hyperreactivity seen here can be ascribed to the presence of such damage.

The lesioned animals were regularly, but not continuously observed during recovery from surgery. During this time, none of the outward signs of epileptogenic damage (e.g. facial myoclonus, rearing, forelimb tremor) were observed. On balance, postulating the presence of epileptogenic damage seems plausible, but somewhat redundant; as evidence already exists for amygdala lesions occasionally causing extreme anxiety or reactivity to handling, a kindling-like phenomenon is not a parsimonious explanation.

In spite of the contradictions in past experiments, the amygdala's primary role in emotional behaviour remains unchallenged. Manipulations of the amygdala may produce increments or decrements in particular emotional behaviours, but seldom leave these behaviours unchanged. Adamec and Morgan's (1994) results show that it is becoming increasingly meaningless to talk about the social/emotional

functions of the amygdala as a whole (see also McGregor and Herbert, 1992, and sections 2.1-2.4. There appear to be dissociations of social/emotional function between corticomedial and basolateral divisions, and so future research should also focus upon this as well as the antero-posterior dissociations found by Adamec and Morgan (1994). The implications of the lack of correlation between reversal performance and handling will be dealt with in the general discussion.

7 - EXPERIMENT 3: ACTIVITY LEVELS.

As the response of amygdalectomised animals to capture appeared atypical, it was decided to test the activity levels of these animals. As assessment took place during daylight, no differences were anticipated between lesioned animals and shams (following Lorenzini et al., 1991).

7.1 - MATERIALS AND METHODS:

SUBJECTS:

The same subjects were used here as in experiment one, omitting the six animals with the "large" (0.5 µl) lesions, as these animals were operated upon and tested when the activity box equipment was not available. Ss therefore numbered 24 in all.

APPARATUS:

Levels of activity were measured with an infrared activity monitoring system (Coulborn Instruments inc., Allentown U.S.A.). This consisted of eight boxes under the control of a Viglen Genie PC, connected by an interface. The boxes (approx 70 X 40cm) were bare apart from an infrared sensor embedded in one of the walls, and a metal grid separating the rats from it. The floors of the boxes were flat plastic, covered with sawdust.

The apparatus recorded six variables: the *number* and *duration* of "no movement events", "short-" and "long movement events". A short movement was defined as lasting for less than one second, and a long movement as longer than this. These variables were recorded by the apparatus at thirty second intervals, and totalled every two minutes for the purposes of output.

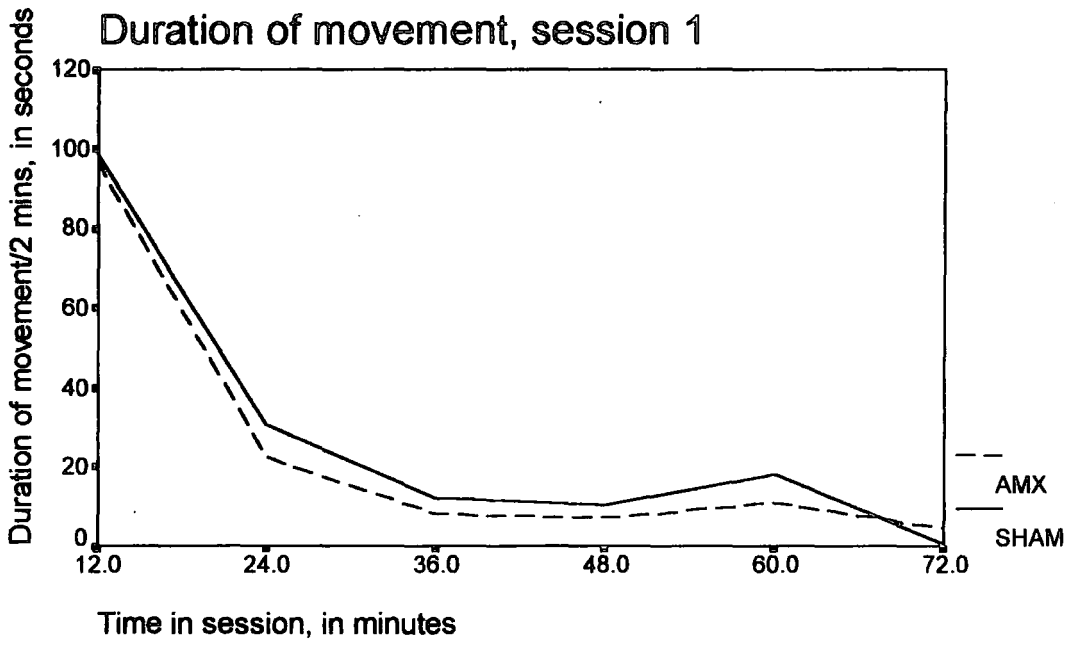
PROCEDURE:

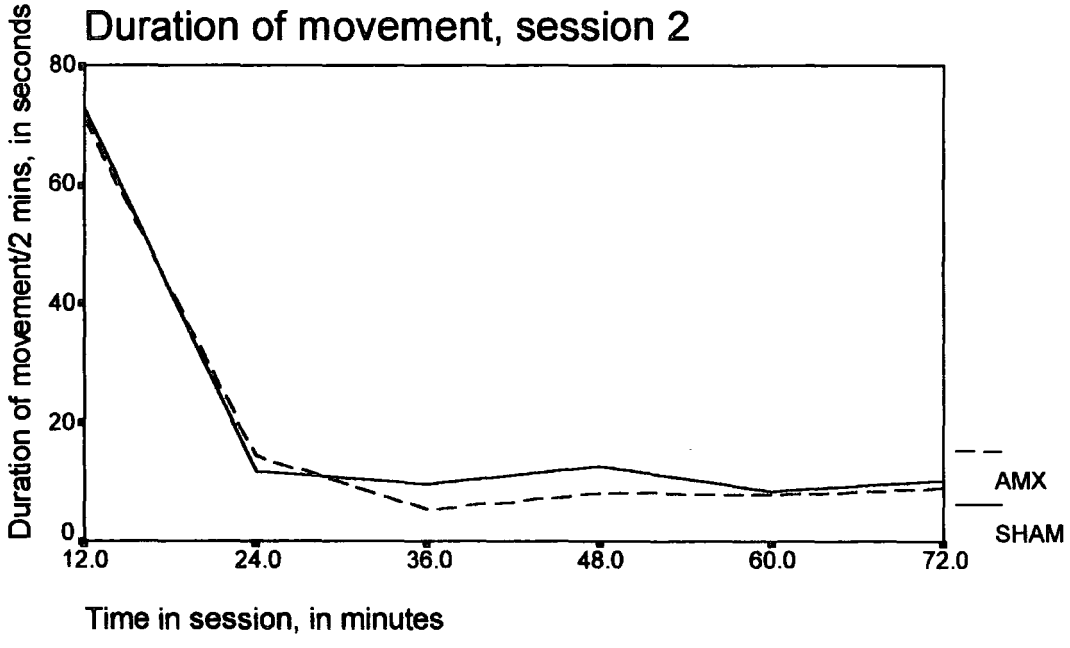
One subject was placed in each box (in counterbalanced order) and left there for 1.25 hours. All tesing was carried out between 9 and 12 am, and Ss were all tested twice over three days (2 sessions/day, 8 Ss/session). The boxes were located in a room that was unfamiliar to Ss, and this room was left empty and undisturbed for the entire testing period. The Ss were not habituated to the boxes or room before testing.

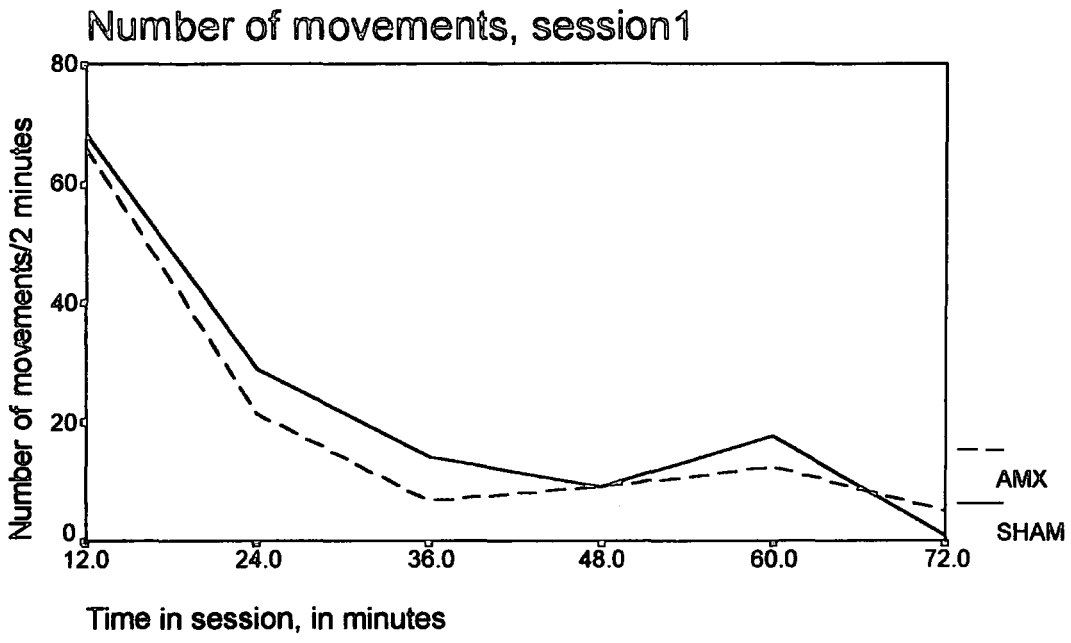
7.2 - RESULTS:

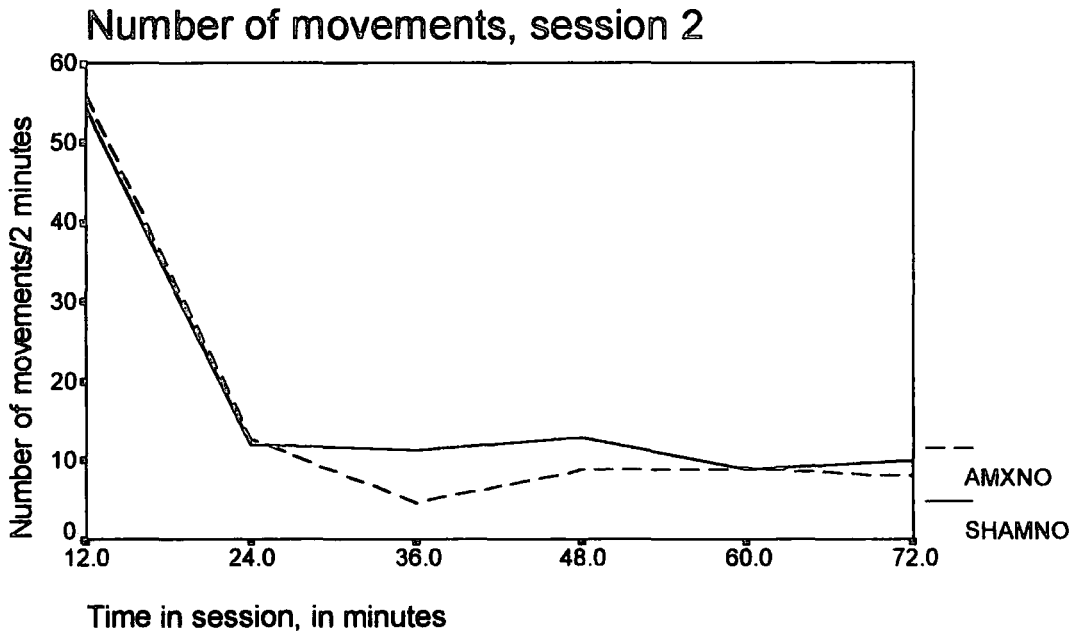
This experiment yielded a large amount of raw data (10656 readings in all), so a number of measures were undertaken to make this more manageable. The original six variables in the output were converted to two - the number and duration of movement events, combining the "short" and

"long" movement variables. Only half of the data was used (every other 2-minute summation), and the two variables were summed at twelve minute intervals; ultimately, both variables were available for analysis at six points during the 1.25 hours (12 minues, 24 minutes, etc.). The graphs below depict the results for duration and time variables for the two testing sessions.









All of the four conditions show a very clear drop in activity over time - $p<0.001$, f 5,90=from 42.45 to 65.72. None show a significant lesion effect:

Condition	**F - value**	**P - value**
Duration, session 1	0.07	0.798
Number, session 1	0.41	0.530
Duration, session 2	<0.01	0.952
Number, session 2	0.05	0.825

There is also no interaction between lesion and time, a critical measure for this issue. P-values for the interaction vary between 0.838 and 0.964. The lesions do not appear to have influenced activity levels in any way.

7.3 - DISCUSSION

These results confirm that amygdalectomy does not cause any gross change in locomotor activity during daylight. The possibilities of an amygdaloid involvement in the regulation of circadian rhythms or the circadian distribution of locomotor behaviour as suggested by Lorenzini et al. (1991) remains open.

Clearly, the changes in emotional behaviour seen in experiment two do not effect quantity of locomotor behaviour. This helps narrow our conception of these changes; locomotor behaviour appears unchanged, quantitative feeding also appears not to have altered (no difference in weights between groups - see experiment two), and general observation of these rats did not reveal any differences in behaviours not related to handling, or threat of handling.

Predictions of changes in neophobia or exploratory behaviour seem to follow more readily from experiment two's results than changes in locomotor behaviour. Past experiments have found amygdalectomised animals to be less neophobic and to explore more than controls (Dunn and Everitt, 1988), although the data from experiment two might suggest that the opposite would be found in these rats. Putting animals into an unfamiliar environment (e.g. the activity boxes) can be construed and a kind of exploration test; if this is so, then Dunn and Everitt's result is not replicated. The boxes permitted little opportunity for detailed exploration, however, as they resembled uniform flat grey plastic buckets - so there are few grounds for doubting Dunn and Everitt's results.

As the role of the amygdala in basic motor function is minimal, and previous results predict no change, these results are not surprising. It still remains of interest, however, to formally test neophobia and exploration levels in this batch of animals, given their altered emotional state.

8 - GENERAL DISCUSSION

This section will be structured as follows. First, I will consider the implications of the results of all of these experiments together, asking what mechanisms might have caused them, and suggesting possibilities for future replications and research. The discussion will then focus on the title of the thesis, and I will attempt to delineate the role of the amygdala in the perception of reward. Past studies and the results of this experiment will inform this discussion, and the "stimulus-reward" theory of amygdala function will be considered afterwards.

In order to consider all three experiments together, the rationale for each experiment must be considered. Experiment one was, of course, the main study, and experiment two was initiated as a response to the experience of handling the lesioned animals. After experiment two yielded positive results, it was decided to test activity levels to try and see if the reactivity to handling was part of a broader change in behaviour.

Experiment three replicated Lorenzini et al (1991), showing no alteration in levels of activity. Experiment one (second part of stage one - also stage two) produced entirely the opposite effect to the anticipated reversal deficit, and stages two and three failed to show the expected effect due to altered secondary reinforcers or changed magnitude of reward. The overall picture appears to show nonreplication (experiment one, stage three) alongside results that are completely the opposite to those anticipated (experiment one stage one, experiment two). One previous study, suggests the presence of some order in these results, however; Aggleton and Passingham (1982) suggest that "both the hypoemotionality and the successive reversal deficit arise from the same underlying dysfunction". Emotionality and reversal performance are also superficially associated in these results (albeit with both variables going in the opposite direction to that anticipated) but detailed correlations (see section 6.2) do not bear this idea out. To summarise, and describe the behaviour produced by these amygdala lesions: the lesioned rats had a low startle threshold, and showed extremely aversive reactions to handling. They performed better than shams at later reversals in a position discrimination task, but were not differentially sensitive to experimental manipulations designed to test sensitivity to secondary reinforcers or shifts in reward magnitude. The emotional and cognitive changes seen did not effect the overall levels of movement recorded.

The counterintuitive nature of these results has already been noted, and the fact that unexceptional, histologically verified amygdala lesions cause this behaviour is startling. The finding of hypoemotionality in amygdalectomised animals has generally been regarded as a result quite as firm and replicable as conditioned taste aversion was before Dunn and Everitt (1988). It does less violence to our preconceptions, however, to imagine a lesion causing an increase in reactivity to handling than to imagine one causing an increase in reversal performance. The analogy of the "hypergnosic" patient has already been mentioned in section 5.31; to make sense of these results, clear replications are needed, along with detailed explanations of the mechanism of reversal learning. To invert Wittgenstein (1953)[1], outer behaviours stand in need of inner processes.

Any further studies or replications on this topic will need to respect two sets of naturally occurring divisions; the types of discrimination learning task, and the phylogenetically defined subsections of the amygdala. Jones and Mishkins' (1972) study used object and spatial discriminations to elegantly dissociate the functions of three temporal lobe areas, and this distinction should be compounded with testing simultaneous and successive discriminations separately. (N.B. the discrimination task in experiment one is referred to as a "position" discrimination, rather than spatial, to be theoretically cautious.) The difference in effect size between simultaneous and successive tasks found in the meta-analysis (section 4.2) points to the fact that this may be an important distinction. So:

Object/Simultaneous	Object/Successive
Spatial/Simultaneous	Spatial/Successive

There is absolutely no *a priori* reason why any of the these four tasks should utilise the same cognitive/neural mechanisms as any of the others. The need to respect the anatomical distinctions of the amygdala has been laboured elsewhere; specificity of lesion site is a problem, but much could perhaps be learned from Holland and Gallagher's laboratory, as they appear to be able to lesion the central nucleus consistently and selectively.

[1] "inner processes stand in need of outer criteria"

Stage two of the experiment offered the appealing possibility of testing Everitt and Robbins' (1992) and Gaffan's (1992) theories in a single task, and deciding between them. The equivocal results obtained mean that the less ambitious task of simply replicating these experimenters' results is now most important. Amygdalectomised rats must be exposed to further tests of sensitivity to secondary reinforcement, and preferably ones substantially different to those summarised in Everitt and Robbins (1992) in order to test the generalisability of their findings. Gaffan's theories were always under less threat from these results, but one experiment in particular merits replication using rats - Gaffan, Gaffan and Harrison (1989). In part of this study, it was shown that amygdalectomised animals perform poorly when the reward is delivered to a location distant from the discriminanda (and thus a visual-visual association cannot be formed between them - see section 3.32). This is easily replicable in a modified operant chamber in which the pellet delivery tray is situated in the wall opposite the levers.

The magnitude of reward task included in stage three demands repetition, as the prediction of a deficit follows so strongly from past experiments. One possible reason for the absence of a deficit is the low criterion which the animals had to reach before reversal; the five "free" trials made the criterion effectively 77.5%. This may not have been challenging enough to reveal the problems that the lesioned animals may have been having. An alternative may be to remove "free" trials altogether, or simply to raise the formal criterion to 100% (effectively 87.5%) - any intervention to increase the criterion level. If amygdalectomised animals performed at normal levels on this modified task, it would present a serious challenge to a simplistic hypothesis of a "magnitude of reward" deficit.

Aggleton and Passingham noted in 1982 that the presence of heightened aggression in one of their amygdalectomised monkeys was a cause for concern, as the lesion was similar to those sometimes used for psychosurgery. The fact that, on average, the entire group of lesioned animals here was more reactive to handling than shams is perhaps even more worrying. Two tests are immediately pertinent; a formal test of startle threshold (following M. Davis' work - e.g. Davis 1992), and a test of anxiety such as the elevated plus-maze. These two tests might confirm the experimenter's unquantified observation of the changed startle response, and replicate Robinson's (1963) findings using a more modern test for anxiety. After these, there is a myriad of potential social/emotional

behaviour findings begging to be replicated, but the most clinically relevant would be measures of aggression. Psychosurgical manipulations are sometimes directed at the amygdala in order to control aggression or rage (for example Lee et al., 1988); any evidence that suggests that such operations may have negative, unanticipated effects must be examined closely.

What is the role of the amygdala in the perception of reward? Or, to put it more concretely, in conditioning to reward? Section 3.3's review of past literature addresses this question - I will now attempt a unitary answer. As an organising principle, I will take Cahill and McGaugh's (1990) proposal that the amygdala's role in appetitive conditioning is minimal compared to its mediation of aversive conditioning. Citing a summary of some of the appetitive experiments in the literature (along with the studies reported in this thesis), I will mount a qualified challenge to this view as well as listing the reward-motivated tasks that the amygdala is involved in.

Cahill and McGaugh (1990) describe a number of tasks, some of which require only one trial to learn, which elegantly show that amygdalectomised rats (for example) learn to find and return to food in a y-maze as quickly as shams, but do not learn to avoid footshock as well as controls. They cite clear dissociations between the two situations, and show that the size of this dissociation appears to increase with the aversiveness of the aversive stimulus. On the basis of this evidence, Cahill and McGaugh (1990) conclude that "it may be that the participation of the amygdala in learning depends upon the degree to which the training conditions induce phasic increases in arousal associated with the release of stress-related hormones." They concede that the amygdala may have some role in appetitive conditioning, but still claim it will be strongest when arousal is highest, and they therefore reject a simple version of the theory that the amygdala mediates stimulus-reward associations.

Cahill and McGaugh (1990) are clearly correct in pointing out that the abundant literature on fear conditioning (see section 3.21 - 3.24, also Davis 1992, 1994, Ledoux 1992 etc) is rather more clear and coherent than that on appetitive tasks (see, for example, section 4.1 on discrimination learning). A quick survey of the appetitive literature, from Holland and Gallgher's work on the mechanisms of classical conditioning to instrumental discrimination tasks, shows that the evidence for amygdala involvement is far from sparse. Starting at the classical conditioning end of the literature, Holland and Gallagher's (section 3.34) sophisticated work on attentional processing clearly

shows one of the functions of the central nucleus, but in a paradigm outside simplistic notions of stimulus-reward associations. The appearance of the visual CS in this paradigm does not presumably activate too many "stress-related hormones", and nor presumably does the basic conditioned cue preference (CCP) paradigm. Although Cahill and McGaugh (1990) find no effect of amygdala lesions on their appetitive CCP, but a clear effect on the aversive version, it must be noted that these results are in complete contrast to the majority of the other results in the literature. McDonald and White (1993), Hiroi and White (1991) and Everitt et al (1991) all record a clear deficit on appetitive CCP in animals with amygdala lesions. Selden et al (1991) record no effect of lesion on an aversive CCP to footshock, and Sutherland and Mc Donald (1990) also show that amygdalectomised animals respond normally to aversively conditioned contextual cues. This inversion of Cahill and McGaugh's results produces problems for their theory, of course. The "arousal/stress hormone" theory can still be tested within the appetitive CCP literature, however. The author does not wish to underestimate the arousing properties of McDonald and White's (1993) Froot Loops cereal for a hungry rat, but presumes it to be less than that of Hiroi and White's (1991) amphetamines. Both of these CCP experiments were performed in the same laboratory. It is indeed the case that the ratio of time spent in the S+ to S- chambers is higher in the amphetamine CCP ($F_{1,49}=1.3$, $p>0.05$) than for the food ($F_{2,21}=0.31$ $p>0.05$), but this confirmation looks redundant in the light of Selden et al's result.

At this stage, we can already assess Cahill and McGaugh's theory - it may be true in some cases (and their evidence is compelling), but if results as clear as the CCP and Holland and Gallagher's work are present, then the theory is so weak or inappropriate in these cases to be uninteresting. Even having reviewed only these two areas, there are more than enough intriguing precedents to justify further research into the amygdala's role in appetitive conditioning - and there are already indications that this role might be more diverse and heterogeneous than it is in the aversive literature.

Section 3.33 describes the work of Everitt, Robbins, Burns et al, which clearly demonstrates the amygdala's role in secondary reinforcement and acquisition of a new response with conditioned reinforcement. It also highlights the essential importance of the amygdala's connections with ventral striatum for the perception of reward. Gallagher and Holland (1994) have recently refined the

localisation of one of these functions, showing that central nucleus damage has no effect on second-order conditioning. Dopamine also features as important in this work, Everitt and Robbins (1992) highlighting the role of the mesolimbic (A10) dopaminergic neurons in the ventral striatum that terminate close to fibres arriving from the amygdala. Dopamine within the amygdala also appears to be important for discrimination learning - Hori et al (1993) compared levels of extracellular dopamine and its metabolites in two groups of rats, one of which had learned a discrimination task. Concentrations of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid increased significantly during the learning sessions in the "discrimination" group, and levels in this group ended significantly higher than in the control group.

Peinado-Manzano (1990, 1994) has reported two experiments in which amygdala lesions disrupted learning of a task with visuotactile discriminanda. In 1990, she found that such lesions disrupted performance of a DNMS task - but left a spatial DNMS task untouched. The 1994 study involved a simple association paradigm - rats were forced to run to both the S+ and S- arms of the T-maze twice each, and then allowed to choose. These results show that the nature of the discriminanda may matter critically in whether or not an amygdalectomised animal can perform a task.

The role of the amygdala in discrimination learning, and also in magnitude of reward tasks, has been detailed at length in sections 4 and 3.31 respectively. Adding these to the work described above, I feel that I have given a fair overview of the main areas of appetitive amygdala unction discovered in the rat so far. A question worth addressing now is - does all of this data confirm the theory that amygdala lesions disrupt stimulus-reward associations across modalities?

Superficially, the theory has much to recommend it. It explains how amygdalectomised animals can successfully perform such tasks as win-stay and win-shift (McDonald and White 1993) and spatial DNMS (Peinado-Manzano, 1990) - these tasks can presumably be performed by using a rule, rather than associating a previously neutral stimulus with a reward valency. The theory also offers the appealing possibility of a unitary explanation for deficits in conditioned fear, discrimination learning and CCP. It is possible that the depth and explanatory capacity of the theory constitute a problem, however; the theory may be a little simplistic, and rather too powerful. The picture engendered by the theory is that of a "reward-agnosic" rat, which sees its world as devoid of value, or

at least rather pallid. Wittgenstein (1953, no.422) would invite us to try and find the application of this picture, and it is a productive exercise to try and imagine the range of tasks at which our reward-agnosic rat would display an impairment. The number of tasks imaginable is enormous, and even begs the question of whether such a rat could perform a (so-called "rule-based") DNMS, when it made no connection between the discriminanda and reward on the initial, forced run. There is a danger of allowing a slight explanatory laziness into the literature here - an experimenter can devise any one of a huge number of appetitively motivated tasks, find a deficit in the performance of amygdala-lesioned animals, and slightly too rapidly ascribe this to problems of stimulus-reward associations. Such problems can be used to explain both magnitude of reward deficits and problems acquiring CCPs, but it is questionable whether it is helpful to do so; a much more engaging line of research seems to be the dissociation between explicit and contextual cues found by Selden et al (1991), which may tell us something entirely new about the nature of the deficit rather than limply confirming an existing theory.

Selden et al's results warn us against assuming that results in the appetitive and aversive literatures are comparable. Quite apart from Cahill and McGaugh's (1990) objections, there are no appetitive equivalents of conditioned taste aversion (CTA) or fear-potentiated startle. Given that CTA and aversive CCP are untouched by amygdala lesions, there is no question of extending results from the aversive literature to try and bolster the stimulus-reward theory in the appetitive literature.

There are problems of specificity in the appetitive literature. It seems highly likely that the nature of the discriminanda used in a task effects the level of performance shown by amygdalectomised animals on that task. Peinado-Manzano's dissociation of spatial and visuo-tactile DNMS has already been cited, as has the contextual/explicit dissociation in the aversive literature. Perhaps Cahill and McGaugh's (1990) success in inducing an appetitive CCP in lesioned animals was due to their unique use of odour as a cue. There is no *a priori* reason to assume that amygdalectomised rats should respond comparably to visual or olfactory discriminanda, particularly as the pattern of projection from the relevant sensory organs to the amygdala is so different.

A theory suggested by Cador et al (1989, amongst others) is that the amygdala plays no role in the simple association between stimulus and reward, but is critical in mediating second-order

conditioning. The experiments described in section 3.33 demonstrate the role in secondary reinforcement clearly, and there are strong parallels with the monkey literature (Gaffan and Harrison 1987). In both monkeys and rodents, deficits in second-order conditioning are clear and well-replicated, and this function appears to be clearly localised in the basolateral division, following Gallagher and Holland's (1994) elimination of the central nucleus. the assertion that the amygdala appears to have no involvement in simple stimulus-reward associations appears to be wrong, however. Something must be causing the problems in discrimination learning and CCP, for example. We can conclude that the amygdala has a clear, if only partially mapped, role in second-order conditioning, and a role in simple conditioning that is not adequately described by the label of "stimulus-reward association problems". We must begin to pay respect to the discriminanda used in "simple" conditioning tasks (why *should* conditioning to environmental cues in CCP involve the same systems as conditioning to a lever in a skinner box?), and perhaps also adopt a more sophisticated approach such as that recommended by Peinado-Manzano (1990): "We suggest the necessity of further experiments dissociating (1) the recognition of sensorial stimuli and its distinction from unfamiliar stimuli (2) their association with their reinforcing meaning (3) the short-and long-term retention of this association, and (4) the inversion of reinforcement contingencies".

In the spirit of this quotation, we must resist two tempting theoretical presumptions about the amygdala's role in the perception of reward; that there will be a single function, and that it will be simple. The deficits in magnitude of reward tasks, for example, may or may not reflect similar processes to those involved in simple discrimination tasks. The consistency of the magnitude of reward literature is markedly greater than that of the discrimination literature, implying if anything that this is not the case. Only further experimentation can resolve this question, and only when motivational factors have been entirely controlled for.

Holland and Gallagher's (1992, also Gallagher and Holland 1994) research is in many ways a paragon of the direction in which future investigations might aim. They focus on a single amygdaloid nucleus. They use an unorthodox set of tasks to attempt to elucidate a deficit which is clearly outside the main tradition of amygdala research, and they use their animals' deficits to "deconstruct" some very basic classical conditioning processes into their component parts. The general impression on

surveying the field is of a group of people who were expecting a ZX-81, but who have instead found a Powermac. The move towards recognising the complexity of the phenomenon at hand is well underway in most laboratories, but it is still important to reject any simple view of the amygdala's role centering around vaguely-defined "stimulus-reward associations" as a retrograde step.

Finally, the results of the experiments reported in this thesis must be set alongside the conclusions reached above. The handling data, although initially surprising, turned out to be consistent with some other results, and probably caused by subtleties of lesion location. The amygdala's role in emotional/social behaviour was affirmed here, however. The rapid reversals of the lesioned animals remain something of a mystery, and cry out for replication; and although the opportunity of deciding between Gaffan and Everitt & Robbins at a single stroke was tempting, it will have to be done more methodically. Everitt & Robbins' research to date is clear and exciting, and needs to be extended. The magnitude of reward deficit seen in amygdalectomised animals has been so consistent in the past that the lack of a deficit here is ascribed to task design. The lesioned animals showed no change in gross levels of activity, and this, at least, is consistent with past results.

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APPENDIX A

Table of studies used in the meta-analysis (section 4.2)

STUDY A:

STUDY NAME AND DATE: Eichenbaum, Fagan and Cohen (1986)

NUMBER OF ANIMALS INVOLVED: 5 amygdalectomised, 5 normal controls, 2 sham controls

BEHAVIOURAL PROCEDURE USED: Learning of three separate olfactory go-nogo discriminations , followed by the reversal of the third pair.

RESULTS, AND EXACT TEST STATISTIC: Trials to criterion on initial discrimination, reconstructed from graph of errors to criterion on p 1880, tested with Mann-Whitney U due to small *n.* Criterion was 18/20 trials correct, or 400 trials/day. p=0.4649.

LESION PLACEMENT, METHOD, AND EXTRAAMYGDALOID DAMAGE: Electrolytic. Described as consistently ablating lateral and "medial basal" nuclei. This is confusing; reconstructions show that the medial sections of both the basal and accessory basal ("basomedial") nuclei were damaged. No extraamygdaloid damage described, bizarre, isolated dorsal white matter damage recorded on reconstructions, however (and ignored in this analysis. Assumed to be a mistake). See p1879.

OTHER NOTES:

STUDY B:

STUDY NAME AND DATE: Freeman and Kramarcy, 1974

NUMBER OF ANIMALS INVOLVED: 5 amygdalectomised, 10 sham, (10 hippocampectomised).

BEHAVIOURAL PROCEDURE USED: Tone discrmination in skinner box, criterion was 4 S+ responses to 1 S- response in a session, or 31 days. Training was postoperative.

RESULTS, AND EXACT TEST STATISTIC: Days to criterion, p=0.0081

LESION PLACEMENT, METHOD, AND EXTRAMAYGDALOID DAMAGE: Electrolytic. Described as primarily in the cortical nucleus, sometimes extending to the basal and medial nuclei. Extraamygdaloid damage not reported, but obviously includes most adjacent cortex on the ventral surface of the brain (see reconstruction, p613).

OTHER NOTES:

STUDY C:

STUDY NAME AND DATE: Han and Livesey, 1977

NUMBER OF ANIMALS INVOLVED: 9 amygdalectomised, 10 shams, (10 hippocampectomised)

BEHAVIOURAL PROCEDURE USED: Simultaneous brightness discrimination in a unique box, which separates stimuli from animals with a transparent door that can be lowered to allow the animals access to respond. Only the "non-enhanced" condition was used here, in which both stimuli disappeared as soon as a response was made. This was judged to be the most similar to experiemtn one.

RESULTS, AND EXACT TEST STATISTIC: Trials to criterion, p=0.364

LESION PLACEMENT, METHOD, AND EXTRAAMYGDALOID DAMAGE: Electrolytic. Centred around basolateral nucleus, averaged about 50% destruction of this structure. No extraamygdaloid damage described or apparent in reconstruction.

STUDY D:

STUDY NAME AND DATE: Kemble and Beckman, 1970

NUMBER OF ANIMALS INVOLVED: 11 amygdalectomised, 8 sham (4 anaesthetic only, 4 anaesthetic + needle).

BEHAVIOURAL PROCEDURE USED: T-maze, spatial/position discrimination. Criterion 9 out of 10 trials correct, then reverse.

RESULTS, AND EXACT TEST STATISTIC: Number of errors to first criterion, p=0.0002

LESION PLACEMENT, METHOD, AND EXTRAAMYGDALOID DAMAGE: Electrolytic. Lesions were large, described as including extensive damage to lateral, basal, central and cortical nuclei. Medial nucleus was spared. All lesion included damage to ventral pyriform cortex, and "frequently" to the claustrum.

OTHER NOTES:

STUDY E:

STUDY NAME AND DATE: Kentridge, Shaw and Aggleton, 1991.

NUMBER OF ANIMALS INVOLVED: 7 amygdalectomised, 7 sham.

BEHAVIOURAL PROCEDURE USED: Object discrimination in a Grice box, criterion 87% correct.

RESULTS, AND EXACT TEST STATISTIC: Trials to criterion, p-value inferred from statement that "t<1". t assumed to be 0.9, p=0.1922.

LESION PLACEMENT, METHOD, AND EXTRAAMYGDALOID DAMAGE: Ibotenic acid. Lesions described as "consistently located in the medial and ventral half of the amygdala", damaging medial and cortical nuclei, variable damage to basal and central nuclei. Lateral nucleus spared. Extraamygdaloid damage described as "very minor", but reconstructions suggest some medial ventral cortical damage.

OTHER NOTES:

STUDY F:

STUDY NAME AND DATE: Peinado-Manzano, 1988

NUMBER OF ANIMALS INVOLVED: 18 amygdalectomised, 12 shams

BEHAVIOURAL PROCEDURE USED: Skinner box go-nogo discrimination to bright/dim lighting. Criterion was 85% responses correct during two consecutive days.

RESULTS, AND EXACT TEST STATISTIC: Using animals trained postoperatively only, the variable used is sessions to criterion, p<9*10^-5, rounded up to that figure.

LESION PLACEMENT, METHOD, AND EXTRAAMYGDALOID DAMAGE: Ibotenic acid, centered around the central nucleus in half of the animals, lateral in the other half. Little evidence of

any extraamygdaloid damage. Some of the central nucleus lesions may extend ventrally slightly into the caudate.

STUDY G:

STUDY NAME AND DATE: Pellegrino, 1968

NUMBER OF ANIMALS INVOLVED: 30 amygdalectomised, 21 controls (9 sham, 12 unoperated).

BEHAVIOURAL PROCEDURE USED: Skinner box go-nogo discrimination to houselight on/off.

RESULTS, AND EXACT TEST STATISTIC: Number of errors to reversal (which occurred automatically after 7 sessions). p=0.5475

LESION PLACEMENT, METHOD, AND EXTRAMAYGDALOID DAMAGE: Electrolytic. Lesions centered around the basolateral group in half of the animals, coricomedial group in the other half. No description of extraneous damage, reconstructions suggect possible minor caudate/cortical damage, but always limited.

OTHER NOTES: The author expressed surprise at the lack of a lesion effect, stating that an effect would have been predicted from his other experiments.

STUDY H:

STUDY NAME AND DATE: Schwartzbaum, Thompson and Kellicutt, 1964

NUMBER OF ANIMALS INVOLVED: 7 amygdalectomised, 6 unoperated controls

BEHAVIOURAL PROCEDURE USED: Skinner box go-nogo discrimination between tones of different pitch. Criterion was 3:1 ratio of S+ to S- responses.

RESULTS, AND EXACT TEST STATISTIC: Deficit in amygdalectomised animals described as "severe", nearly half of the animals never reaching criterion. This study was assigned a p-value of 9*10^-5.

LESION PLACEMENT, METHOD, AND EXTRAAMYGDALOID DAMAGE: Lesions were electrolytic and huge. They mainly spared the corticomedial subdivision, but typically invaded the claustrum, ventral white matter, ventral putamen/globus pallidus.

OTHER NOTES: The authors note that poor performance appeared to correlate with damage to the putamen.

APPENDIX B

Program referred to in section 5.1

```
 10 CLS
 12 MODE 131
 20 KILL ALL
 30 PRINT:PRINT "          -   beth  -"
 40 PRINT "  2 vs *** 1 *** spatial discrimination task"
 50 PRINT:PRINT
 55 PRINT " DATE : ";TIME$
 60 INPUT "Name/number of rat? ";rat$
 70 PRINT
 80 INPUT "Please enter the required number of trials ";total
 85 DIM data$(total),correct$(total)
 90 PRINT
100 INPUT "What is the correct response? ";resp$
105 PRINT "Press any key to start.":a$=GET$
110 PROCinit
113:
115:
240:
250 !(SWITCH A%, ON)PROCright(A%)
260 !(SWITCH B%, ON)PROCwrong(B%)
270 WAIT
280 END
290:
300:
310 DEF PROCright(Q%)
315 IF re<>0 THEN PROCretract:GOTO 330
320 PORT 3=llev+rlev:A=INKEY(10):PORT 3=0
330 A=INKEY(50)
340 PORT 3=rf+tray:A=INKEY(100):PORT 3=tray
350 store=store+1:trials=trials+1
360 IF Q%=1 THEN data$(trials)="L":correct$(trials)="L"
370 IF Q%=2 THEN data$(trials)="R":correct$(trials)="R"
380 A=INKEY(500)
390 IF trials=total THEN PROCend
395 PROCnose
410 ENDPROC
420:
425:
430 DEF PROCwrong(Q%)
440 PORT 3=llev+rlev:A=INKEY(10):PORT 3=0
450 A=INKEY(50):REM neurotic, I know.
455 PORT 3=rf+tray:A=INKEY(50):PORT 3=tray
457 PORT 3=house:A=INKEY(50):PORT 3=0
460 trials=trials+1
470 IF Q%=1 THEN data$(trials)="L":correct$(trials)="R"
480 IF Q%=2 THEN data$(trials)="R":correct$(trials)="L"
490 A=INKEY(500)
500 IF trials=total THEN PROCend
505 PROCnose
520 ENDPROC
530:
540:
550 DEF PROCend
553 pert=TIME
554 PORT 3=0
555 CLS
556 PRINT:PRINT
565 PRINT
566 PRINT "All over."
570 PRINT
580 INPUT "Do you want a printout? (Y/N)? ";hip$
590 IF hip$="N" THEN GOTO 610
```

```
605 PRINT "    2 vs 1 spatial discrimiantion"
607 PRINT
610 PRINT "Rat name/number : ";rat$
620 PRINT "The correct response was the ";corr$;" lever."
630 overall=(store/total)*100
640 PRINT "Percentage correct was ";overall
650 IF overall>=90 THEN did$="did" ELSE did$="did not"
660 PRINT "So this rat ";did$;" reach criterion (90%)."
670 PRINT
680 PRINT "These are the individual responses by trial number. The letter in
ckets is the correct response for purposes of comparison."
700 FOR n=1 TO total
710 PRINT n";";data$(n);"(";correct$(n);") ";
720 NEXT n
721 PRINT
722 PRINT "This rat took ";(pett/100);" seconds"
723 VDU 3
725 KILL ALL
730 END
740:
750:
760 DEF PROCnose
770 PORT 3=0
780 A=INKEY(200)
790 PORT 3=tray+128
800 REPEAT
810 UNTIL SWITCH 0=-1
820 FOR w=1 TO 5
825 IF trials=spec(w) THEN PROCspecial:ENDPROC
827 NEXT w
830 PROCputemout
860 ENDPROC
1100:
1110:
1120 DEF PROCinit
1130 GOVN SWITCH 24 TO 31
1140 FREE SWITCH 1,2
1145 DIM spec(5),choose(total)
1146 FOR s=1 TO total:choose(s)=s:NEXT s
1147 PROCseed
1150 IF resp$="L" THEN A%=1:B%=2:corr$="Left"
1160 IF resp$="R" THEN B%=1:A%=2:corr$="Right"
1180 rf=1:tray=2:house=4:llev=8:rlev=16:llight=32:rlight=64
1190 trials=0:store=0:re=0
1195 *KEY0 PORT 3=24:A=INKEY(10):PORT 3=0|M
1196 *KEY9 |U
1197 *KEY5 PORT 3=0|M
1198 *KEY7 SWITCH ON 27:A=INKEY(10):SWITCH OFF 27|M
1200 A=INKEY(400)
1205 PROCnose
1215 TIME=0
1220 ENDPROC
1230:
1240:
1500 DEF PROCseed
1510 FOR a=1 TO 5
1520 b=RND(total)
1530 IF choose(b)=0 THEN GOTO 1520
1540 spec(a)=choose(b)
1550 choose(b)=0
1560 NEXT a
1570 ENDPROC
1680:
1690:
1700 DEF PROCputemout
1710 A=INKEY(100)
1715 GOVN SWITCH 1,2
1720 PORT 3=24:A=INKEY(10):PORT 3=llight+rlight
1725 A=INKEY(50):FREE SWITCH 1,2
```

```
1880:
1890:
1900 DEF PROCspecial
1910 A=INKEY(100)
1920 GOVN SWITCH 1.2
1930 IF resp$="L" THEN PORT 3=8:A=INKEY(10):PORT 3=llight:re=1
1940 IF resp$="R" THEN PORT 3=16:A=INKEY(10):PORT 3=rlight:re=2
1950 A=INKEY(50):FREE SWITCH 1.2
1960 ENDPROC
2001 REM A SPACE ODYSSEY
2080:
2090:
2100 DEF PROCretract
2110 IF re=1 THEN PORT 3=8:A=INKEY(10):PORT 3=0
2120 IF re=2 THEN PORT 3=16:A=INKEY(10):PORT 3=0
2130 re=0
2140 ENDPROC
```