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Effect of Basolateral Amygdala Lesions on Learning Taste Avoidance Under Various Water Deprivation Schedules

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ABSTRACT

Learned taste avoidance (LTA) was studied by allowing rats to drink a novel sweet solution followed by induction of gastric malaise (training). When the solution was presented again (test), normal rats reduced their consumption. Ultrasonic vocalizations indicated that the rats experienced positive affect during training which shifted to negative affect during the test. Basolateral amygdala lesions eliminated the LTA and the negative affective shift when the rats were 23 hr water deprived during both training and test suggesting amygdala-based Pavlovian conditioning, but only attenuated the LTA and eliminated the aversive shift when the rats were 3 hr deprived on the test, suggesting instrumental learning. When rats were 3 h deprived during training the lesions had no effect on either the LTA or the negative affective shift, suggesting an amygdala-independent form of LTA based on latent learning.

Résumé

Nous avons étudié le phénomène d'aversion au goût associé (AGA – Learned taste avoidance) en présentant a nos rats une nouvelle solution sucrée suivie d'une indiction de malaise gastrique (entraînement). Lorsque la dite solution est présentée une deuxième fois (teste) les rats normaux ont réduit la quantité de fluide consommée. Les vocalisations ultrasoniques des rats démontrent que l'expérience affective positive vécue durant l'entraînement s'est transformée en expérience affective négative durant le période de test. Des lésions de l'amygdale basolaterale ont éliminé l'AGA ainsi que l'expérience affective négative lorsque les rats sont privés d'eau pendant 23 heures lors de l'entraînement et du test suggérant ainsi un conditionnement Pavlovian dépendant de l'amygdale. Cependant le phénomène d'AGA était seulement réduit et l' l'expérience affective négative éliminée lorsque les rats étaient privées d'eau pendant 3 heures seulement lors du test ; ce qui indique une forme d'apprentissage instrumentale. Lorsque les rats étaient privées d'eau pendant 3 heures lors de l'entraînement et du test, les lésions n'ont eu aucun effet sur l'AGA ni sur l'expérience affective, ce qui nous porte à conclure qu'il s'agit ici d'une forme d'apprentissage latent indépendant de l'amygdale.

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INTRODUCTION

As the French say "*quand l'appetit va tout va*", meaning that when you have a healthy appetite all is well. It is an indication of the social and physiological importance of eating. Ingestion of nutrients is also necessary for the survival of any living organism. Eating in mammals is a complex behaviour involving elaborate neural signalling that integrates visceral and external information. Palatable foods, which are usually nutritious, are approached and consumed, while sour and bitter tastes, which are often poisonous, are usually avoided (Delamater, Lolordo, & Berridge, 1986). But evolution has gone even further. If ingestion of a tasty food is followed by gastric malaise, which could be an indication that the food is in fact poisonous or otherwise injurious, and if the individual survives its encounter with such a substance, it will be avoided from that time on. Since this change in behaviour towards the offending substance is due to experience it is considered to be a case of learned avoidance. The generality of this learning process was demonstrated by the discovery that the illness does not have to be caused by the palatable substance – any gastric malaise due to any cause during some period following ingestion is sufficient to produce the learned avoidance. The phenomenon is generally known as conditioned taste avoidance. Because the term "conditioned" is associated with a particular type of learning, the present thesis uses the more neutral term Learned Taste Avoidance (LTA).

In 1955 Garcia and colleagues discovered that rats exposed to ionizing radiation (which produces gastric malaise) after drinking solutions that were novel to them subsequently avoided consumption of those solutions (Garcia, Kimedorf, & Koelling, 1955). Rats were separated into 6 groups that were exposed to various levels of gamma rays (0, 30 and 57 r) and had access to either a palatable saccharine solution or tap water

for 6 hours. Only the saccharine/radiation- paired groups avoided drinking the saccharin solution when it was subsequently presented alone. The lowest level of radiation (30 r) was sufficient to induce avoidance of solutions that tasted of saccharin, however the conditioning was dose-dependent: the higher radiation levels produced a stronger avoidance that persisted 30 days after the radiation exposure. The phenomenon was thought to reflect the association of the aversive effects of the radiation with the taste of the solution, however the effect worked best with an unfamiliar taste as no avoidance was observed following the pairing of water (a familiar taste) and radiation.

LTA is now a widely used model for investigating conditioning and learning, and is often described in terms of Pavlovian conditioning. According to this view a neutral or palatable taste (conditioned stimulus –CS) is avoided following the presentation of an aversive agent such as radiation, lithium chloride injection, or other emetic agent (unconditioned stimulus - US) that naturally produces an unconditioned response (UCR), gastric malaise in this case. After the pairing the CS acquires the property of eliciting a conditioned response (CR) that is similar to the UCR. The conditioned aversive response results in reduced consumption of the palatable solution.

Learned taste avoidance has been observed in different species, from birds and reptiles to mammals (Barber, Klunk, Howorth, Pearlman, & Patrick, 1998). In humans taste avoidance learning can influence food choices. Taste avoidance is a common effect observed in cancer patients undergoing chemotherapy (Boakes, Tarrier, Barnes, & Tattersall, 1993; Boakes R.A. , 1993; Montgomery & Bovbjerg, 1997; Riley & Freeman, 2004; Tomoyasu, Bovbjerg, & Jacobsen, 1996). In some cases the negative effects of cancer treatments, which include LTA, are severe enough to prompt patients to discontinue treatments (Limebeer, Hall, & Parker, 2006). In addition cancer patients can

also display anticipatory nausea and vomiting (Boakes et al., 1993; Boakes R.A. , 1993; Limebeer et al., 2006) . Taste aversion learning has also been used for ecological control (Nicolaus, Crowe, & Lundquist, 1992).

Hedonic Shift in Learned Taste Avoidance

A fundamental property of learned taste avoidance is a change in CS consumption that is assumed to be the product a hedonic shift in a negative direction. This shift is most obvious when the CS is a palatable solution that is preferred to water, making the observed avoidance following CS-US pairing all the more striking. This hedonic shift implies a change in the affective state produced by the CS from reward to aversion, which leads to common term for this phenomenon: conditioned taste aversion.

The hedonic properties of reward and aversion are affective states that cannot be directly observed but must be inferred from behaviours that are observable. The most common such inference is based on approach and withdrawal behaviour. If an organism approaches and avidly consumes some food we conclude that it "likes" the food, by which we imply that the food produces a rewarding or positive affective state. If an organism avoids food or any other stimulus we conclude that it does not like that food, implying that it produces an aversive hedonic state. This section reviews two other measures of affective states and their use in LTA studies.

Orofacial and Somatic Responses

Orofacial responses (ORs) are an innate set of facial expressions produced in response to palatable or aversive tastants. These behavioural observations have been used to infer hedonic or affective value based on specific forms of these responses when substances with universally agreed-upon hedonic attributes are applied to the mouth. On this basis it has been suggested that facial expression patterns reflect a 'core hedonic

process' of palatability or affect (Berridge, 2000; Berridge & Schulkin, 1989; Delamater et al., 1986).

Grill and Norgren (1978a, b) discovered these stereotyped responses after infusing various substances directly into a rat's mouth and analyzing its facial expressions. Sapid stimuli such as sucrose and low concentrations of salt elicited a response sequence beginning with low amplitude, rhythmic mouth movements, followed by rhythmic tongue protrusions along the midline, lateral tongue movements (licking the lips) and paw licking in rats. Aversive substances such as quinine elicited a response pattern beginning with head shaking; chin rubbing and gaping responses (rapid, large-amplitude opening of the mandible) paw shaking & pushing, chin rubbing, head shaking and face washing (Grill & Norgren, 1978a, 1978b). These sets of reactions were categorized as ingestion and rejection responses respectively and are highly consistent within and between rats.

Similar facial responses are observed across a wide range of species from human infants to other primates to rats (Berridge, 2000; Parker & Carvell, 1985). Travers and Norgren (1986) suggest that the muscular movements involved in the gaping response in rats mimic those involved in vomiting, although rats are actually unable to vomit (Delamater et al., 1986; Parker & Limebeer, 2006).

Parker and her group examined the ingestion and rejection orofacial and somatic reactions in response to learned taste avoidance (Parker 1982,1991). Although intraoral infusions of sucrose normally elicit facial responses associated with reward (tongue protrusions and paw licking), infusions of sucrose after pairing with LiCl injections elicited aversive taste reactions (gaping, paw threading and chin rubbing) (Parker 1982,1991). These results were replicated in a study where rats were allowed to actively

ingest palatable foods as opposed to passively being fed. Parker and Jensen (1992) allowed animals to eat very palatable food within a specific environment after which they were injected with LiCl. Subsequent exposure to the food within the testing environment caused the rats to produce aversive facial responses (chin rubbing, paw treading and gaping) and they did not consume the food (Parker & Jensen, 1992).

Ultrasonic Vocalizations

Myomorph rodents, including rats, emit broadband vocalizations composed of audible (>20 kHz) and ultrasonic frequencies (<20 kHz) in response to various social and environmental situations. The audible vocalizations are believed to be the product of vocal chord vibrations whereas the ultrasonic calls are likely produced by vibrations of the laryngeal folds in whistle-like manner (Roberts, 1975). The ultrasonic vocalizations (USVs) produced by rats vary between 20 and 70 kHz. The frequency range of rat USVs changes over time as a function of development. During early postnatal development pups produce vocalizations predominantly around 40 kHz whereas adult rats can produce at least two (~22KHz and ~50KHz) different types of ultrasound vocalizations identified by differences in frequency and duration (Sokoloff, Blumberg, Whishaw, & Kolb, 2005).

Adult rat vocalization is composed of at least two qualitatively different characteristic calls that differ in their frequency range, modulation and duration. The 22 kHz vocalizations (18-32KHz) are referred to as "long calls" can vary from 300 to 3000ms. These calls are mainly observed in connection with aversive events (after defeat in aggressive encounters, following exposure to predators, termination of foot shock or during withdrawal from substance abuse) (Blanchard, Yudko, Blanchard, & Taukulis, 1993; Borta, Wohr, & Schwarting, 2006; Brudzynski, Bihari, Ociepa, & Fu, 1993;

Burgdorf, Knutson, Panksepp, & Shippenberg, 2001; Covington & Miczek, 2003; Sokoloff et al., 2005).

The other form of rat USVs are of shorter duration (<80 millisecond) and are of higher frequencies (37-70 kHz). These calls are associated with positive events or high arousal situations such as rough-and-tumble play, experimenter-induced tickling, copulation as well as in response to amphetamine administration (Brudzynski, 2007; Burgdorf J, 2000; Knutson & Burgdorf, 2002; Panksepp, 2007; Panksepp & Burgdorf, 2000, 2003).

Using high frequency recording techniques both frequency modulated calls (such as those just described) and non-frequency modulated calls have been detected (Burgdorf, 2005). The non-frequency modulated calls in the 50 KHz range did not seem to be associated with appetitive behaviours; in the 22 KHz range the non-frequency modulated calls occurred primarily in submissive male rats during aggression whereas frequency-modulated calls occur primarily after males ejaculate during mating (White, Gonzales, & Barfield, 1993). More research is needed in order to understand the function of the non-frequency modulated calls.

The Function of Ultrasonic Vocalizations

Ultrasonic vocalizations have been detected in wild rats, laboratory rats living in colonies as well as in isolated laboratory rats (Knutson, Burgdorf, Panksepp, & Vol, 2002). Initially they were thought to be simple by-products of locomotion causing thoracic compression and resulting in a vocalization. This theory has been disproved since rats can produce ultrasonic calls in the absence of movement or during minimized locomotion (Burgdorf et al., 2001; Knutson & Burgdorf, 2002; Panksepp & Burgdorf, 2000, 2003; Sokoloff et al., 2005)

Another explanation of USV function attributes the pup vocalizations to the neonate's method of achieving behavioural thermoregulation before it develops physiological thermoregulation (Blumberg & Sokoloff, 2001; Blumberg, Sokoloff, Kirby, & Kent, 2000). This theory stems from observations of infant rat vocalization eliciting maternal retrieval. This behaviour stops after 12 days of age when the pups stop relying on their mother and littermates for warmth (Blumberg, Efimova, & Alberts, 1992; Blumberg & Sokoloff, 2001; de Ghett, 1978). However an alternative explanation ascribes pup vocalizations to distress calls serving as communication signals (Brudzynski et al., 1993; Knutson et al., 2002; B. Knutson, Burgdorf, & Panksepp, 1999; Panksepp, 2007). The idea that USVs have a communicative role is consistent with the facts that rat ultrasounds are carried over short distances and most natural predators of rodents have limited hearing for ultrasonic noise making them an efficient and evolutionarily functional communication medium (Brudzynski et al., 1993; Knutson et al., 2002; Sewell, 1970).

Chehayeb and Clarke (2007) showed that pairs of rats vocalized more than single rats. Furthermore 1 mg/kg of amphetamine significantly increased 50 kHz USV produced by singly-tested rats, but in paired-test rats smaller doses (0.25 and 0.5 mg/kg) were sufficient to elicit a significant increase in high frequency calls. Amphetamine increased high frequency vocalizations in dose dependent manner until a plateau of 2 mg/kg. Chehayeb et al (2007) also showed context-specific sensitization on 50kHz calls paralleling amphetamine context-specific sensitization.

Consistent with these findings, 6-OHDA lesions of ventral striatum significantly decrease the total number of 50kHz calls. In normal rats pre-treatment with haloperidol (a dopamine D_2 antagonist) also diminishes high frequency calls following an amphetamine

injection thus suggesting a close interaction between the reinforcing effects of the drug and the rate of 50 kHz USVs produced (Ciucci et al., 2007). Reports that rats produce 50 kHz USVs in response to a cue predicting the onset of electrical brain stimulation (Burgdorf, Knutson, & Panksepp, 2000) and during the appetitive phase of sexual solicitation (Floody, Pfaff & Lewis, 1977; Sales & Pye, 1974; White et al., 1998) are also consistent with this idea.

The ontological affective nature of rodent's USVs shows commonalities with other forms of mammalian communication. Mammals share evolutionarily conserved brain circuits that facilitate specific kinds of emotional arousal and social interaction (Burgdorf, Wood, Kroes, Moskal, & Panksepp, 2007; Knutson et al., 2002; Panksepp & Burgdorf, 2003). Some workers (Knutson et al., 2002) have suggested that the commonality of these subcortical structures and corresponding functional homologies means that even mammals with different sensory capacities may experience similar affective experiences. These authors have also suggested that rat USVs may be functionally comparable to human positive activation (PA) (Knutson et al., 2002) as indicators of affective states. In a state of high PA, humans can be described as active, aroused, and happy, while in low PA they are quiet, sleepy, and lethargic. Rat USVs may be regarded as similar indicators. The expressions of these affective experiences differ from one species to the other, making it almost impossible to compare them. However within a species behaviour such as USVs may be a useful measure of affective state given that they can be reliably associated with other measures of affect. A good validation of the use of USVs would be to compare USVs and orofacial responses. At this point there are no reports of such a comparison.

Neural Systems Supporting Ultrasonic Vocalizations

Some evidence suggests that different neural substrates are involved in the production of the two forms of calls, hinting at the possibility of distinct neural systems for each type of call (Burgdorf et al., 2000; Burgdorf et al., 2007). Stimulation of brain areas previously shown to support intracranial self-stimulation (ICSS), such as the prefrontal cortex, nucleus accumbens, ventral pallidum, lateral preoptic area, lateral hypothalamus, ventral tegmental area, and raphé has been shown to induce 50-kHz calls (Burgdorf et al., 2007). Similarly rats emit 50-KHz calls following intracranial injection of DAMGO, a μ–opiate agonist into the ventral tegmental area. The same treatment also produces a conditioned place preference for and increased 50-KHz calls in the environment where the effects of DAMGO were experienced in most rats. Rats that did not emit 50-KHz calls following DAMGO injection also failed to exhibit the conditioned place preference (Burgdorf et al., 2007; Knutson & Burgdorf, 2002). Micro-infusion of the glutamic acid decarboxylase blocker semicarbazide into the central nucleus of the inferior collicuclus, an area involved in aversive behaviour and defence mechanisms abolished the emission of 22-KHz calls during a conditioned freezing task.

Orofacial responses and USVs are methods that can monitor the hedonic shift that takes place during conditioned taste avoidance. As mentioned orofacial and somatic responses have been used and well documented in the study of conditioned taste learning. On the other hand, USVs have never been used in such a setting.

Anatomy of Learned Taste Avoidance

Taste

The processing of conditioned taste avoidance starts with the activation of taste buds in the mouth and is relayed through cranial nerves VII, IX and X to the rostral

nucleus of the solitary tract (NST). The signal is then sent to the parabrachial nucleus (PBN). Two pathways exit the PBN in direction of the forebrain: the thalamocortical path projects to the gustatory thalamus which in turn projects to the gustatory part of the insular cortex (IC). The other pathway leaving the PBN consists of mostly reciprocal projections to the central nucleus of the amygdala (CeN), the basolateral amygdala (BLA), the basomedial amygdala (BMA), the lateral hypothalamus, the bed nucleus of the stria terminalis (BNST) and the zona incerta (Cechetto, 1987; Reilly & Bornovalova, 2005). The projections from the PBN to the IC are believed to pass through the CeN which complicates interpretation of the effects of CeN lesions.

Disrupting Taste Learning.

Damaging the chemoreceptors thus blocking taste detection can impair taste learning. Ageusia (inability to taste) can be achieved by lesioning the cranial nerves, and thus preventing the transmission of taste information to the brain (Mendelson et al., 1974; Reilly & Bornovalova, 2005). However complete ageusia is difficult to attain and is rarely used as a method for studying taste avoidance (Bartoshuk & Beauchamp, 1994). Taste learning can also be disrupted by directly affecting the gustatory cortex and structures involved in conditioning such as the basolateral and the central nuclei of the amygdala (BLA and CeN respectively), the parabrachial nucleus (PBN) and the insular cortex (IC) (Cubero, Thiele, & Bernstein, 1999; Grupp, Linseman, & Cappell, 1976).

Basolateral Amygdala

The amygdala, located in the ventral part of the temporal lobe, is divided into basolateral, central, medial and cortical nuclei (Swanson, 2003; Swanson & Petrovich, 1998). Considerable evidence suggests that the BLA is important for learning involving motivationally significant events (Blundell, Hall, & Killcross, 2001; Tye & Janak, 2007)

and that both appetitive and aversive Pavlovian conditioning is impaired by lesions of the amygdala (Antoniadis & McDonald, 2001; Balleine & Killcross, 2006; Everitt, Cardinal, Parkinson, & Robbins, 2003; Everitt, Morris, O'Brien, & Robbins, 1991; Gallagher, Graham, & Holland, 1990; Grupp et al., 1976; Jones & Mishkin, 1972; LeDoux, 1995; McDonald & White, 1993; Weiskrantz, 1964). Lesions of the BLA impair the conditioned cue preference for food and addictive drugs (Bardo, 1998; Carr & White, 1986; Holahan, 2005; McDonald & White, 1993; Tzschentke, 1999; White, 2004) and also eliminate conditioned freezing (Antoniadis & McDonald, 2000; Killcross, Robbins, & Everitt, 1997; Schafe, Nader, Blair, & LeDoux, 2001). Other studies demonstrate a role for the amygdala in encoding reward magnitude memories and conditioned cue (or place) preference (Carr, Fibiger, Phillips, & Liebman, 1989; Chai & White, 2004; Gilbert, Campbell, & Kesner, 2003; Gilbert & Kesner, 2002). The BLA is also involved in representation of reward value and goal directed behaviour (St.Andre & Reilly, 2007) as well as reward devaluation (Gilbert & Kesner, 2002; Pickens et al., 2003). Overall the amygdala may play a significant role in learning motivationally driven stimulus-reinforcer associations regardless of whether the reinforcer is positive or negative.

Factors Interacting With Amygdala Lesions in Taste Avoidance Learning

Since the amygdala is a key structure in both the gustatory system and in Pavlovian conditioning its role in learned taste avoidance has been the subject of many investigations (Balleine & Killcross, 2006; Blundell et al., 2001; Parkinson, Robbins, & Everitt, 2000; Stouffer & White, 2006; Tye & Janak, 2007). In one of these studies increased c-Fos expression in the BLA was observed following taste avoidance training, suggesting that this structure may participate in taste learning (Ferreira, Ferry, Meurisse, & LÇvy, 2006; Wilkins & Bernstein, 2006). However, the literature is replete with

contradictory effects of amygdala lesions on learned taste avoidance (Dunn & Everitt, 1988; Morris, Frey, Kasambira, & Petrides, 1999; Reilly & Bornovalova, 2005; Schafe, Thiele, & Bernstein, 1998; Yamamoto & Fujimoto, 1991; Yamamoto, Fujimoto, Shimura, & Sakai, 1995; Yamamoto, Fujomoto, Shimura, & Nobuyuki, 1994). These discrepancies may result from differences in experimental procedure such as lesion methods and lesion location, differences in the type of CSs used and conditioning methods, as well as differences in deprivation states during training and testing.

Lesion Location

It is difficult to attribute the effects of amygdala lesions reported in the literature to the BLA only because most lesions extended well beyond the borders of this structure involving both other parts of the amygdala and other structures; researchers often do not target a particular part of the amygdala and/or do not specify which nuclei were affected by their lesions. In cases where these parameters are specified, it is clear that lesions of different nuclei yield different results (Bermudez-Rattoni, Grijalva, Keifer, & Garcia, 1986; Gallagher et al., 1990; Holland & Gallagher, 1993; Morris et al., 1999; Parkinson et al., 2000; Swanson, 2003; Swanson & Petrovich, 1998; Yamamoto et al., 1995).

Medial amygdala lesions have been shown to impair learned taste avoidance (Aggleton, Petrides, & Iversen, 1981; Bermudez-Rattoni et al., 1986; Meliza, Leung, & Rogers, 1981; Schoenfeld & Hamilton, 1981; Yamamoto et al., 1995). The medial amygdala is often overlooked in taste learning studies or is combined with the central amygdala. The exact role of this structure in learned taste avoidance is not well determined.

The CeN receives ascending gustatory and visceral afferents and projects to the PBN and IC (Frey, Morris, & Petrides, 1997; Swanson, 2003; Swanson & Petrovich,

1998) making it a potentially important structure in conditioned taste learning. However there is a discrepancy in the literature concerning the role of this structure in learned taste avoidance. Many studies show that lesions of the CeN do not affect taste learning (Bermudez-Rattoni, Okuda, Roozendaal, & McGaugh, 2005; Reilly & Bornovalova, 2005; Schoenfeld & Hamilton, 1981; St.Andre & Reilly, 2007; Yamamoto et al., 1995) whereas others show the opposite result (St.Andre & Reilly, 2007; Yamamoto et al., 1995; Yamamoto et al., 1994).

St-Andre and Reilly lesioned the CeN using neurotoxic lesions and found that such lesions had no effect regardless of whether the CS was familiar or novel. Furthermore immunohistochemical detection of c-Fos showed no activation in the CeN following saccharin-LiCl pairing (Yamamoto et al., 1994). The c-Fos protein represents an immediate early gene lasting only a few hours after transcription (Dragunow & Faull, 1989; Fenelton, Poulain, & Theodosis, 1993; Sagar, Sharp, & Curan, 1988). Immunohistochemical detection of c-Fos can be correlated to a particular behaviour. However, since c-Fos expression is related to neuronal excitation but not inhibition, is presence can provide evidence that a positive structure is involved in the behaviour, but its absence is not evidence of lack of involvement. Negative results are therefore inconclusive.

Yammamoto's results are in opposition to other findings reported in the literature. Wilkins and Bernstein (2006) measured Fos-like immunoreactivity (FLI) in BLA and CeN after pairing CS taste and US drug using bottle and IO conditioning methods Conditioning using the bottle method led to elevations in FLI in both the basolateral and the central amygdala while I/O conditioning led to activation in the CeN only. It appears that the CeN is only implicated in learned taste avoidance when the rats are trained using

a forced-drinking method regardless of the lesion method used. When rats are trained with voluntary drinking lesioning the CeN has no effect. As in the literature on medial amygdala lesions the histology is difficult to compare as the boundaries of lesions often extend beyond the central nucleus and spill over to neighbouring nuclei (Reilly & Bornovalova, 2005).

Lesion Method

Different lesion methods often produce different results because they create different types of damage. Electrolytic lesions damage both cell bodies and fibres of passage, neurotoxic lesions damage cell bodies and tend to spare fibres. Dunn and Everitt (1988) published a pivotal article comparing the effects of electrolytic and ibotenic acid lesions mainly confined to the basolateral area of the amygdala on learned taste avoidance induced by LiCl. Ibotenic lesions had no effect on the behaviour whereas the electrolytic lesions attenuated the avoidance and accelerated extinction. They concluded that electrolytic amygdala lesions are effective only to the extent that they cause incidental damage to fibres of passage projecting to and/or originating in other structures required for this form of learning. According to this idea, ibotenic acid does not affect taste avoidance learning because it spares these passing fibres.

Similarly, Schafe et al (1998) found that electrolytic but not excitotoxic lesions of the amygdala attenuated the expression of the learned taste avoidance when voluntary drinking was used. The area affected by the lesions was much more restricted to the basolateral area of the amygdala than in the Dunn and Everitt study (1988). This difference between neurotoxic and electrolytic lesions has been confirmed by several other studies (Frey et al., 1997; Reilly & Bornovalova, 2005; St.Andre & Reilly, 2007;

Yamamoto & Fujimoto, 1991; Yamamoto et al., 1995). However these results are not always comparable as the lesioned areas are not exactly similar.

Method of CS Presentation

There are two possible ways of presenting fluid to animals during taste learning. The first method involves involuntary drinking, accomplished by intra-oral (I/O) infusion. A catheter is inserted into the animal's mouth and fixed to its jaw. Fluid is squirted directly into the rat's mouth. Avoidance is measured in terms of rejection latency i.e. the time between the beginning of the taste infusion and the appearance of rejected drops of liquid on the animal's chin. Rats are not necessarily water deprived during training when the I/O method is used. In some studies the I/O method is used to train the animals which are then tested by presenting the CS in a drinking tube (discussed below). Since the rats do not make any kind of voluntary response when they are exposed to a CS presented by the IO method, this paradigm is thought to produce pure Pavlovian conditioning.

The second method involves voluntary drinking in which the rats are given access to one or more drinking spouts. Most workers use water deprivation with this type of presentation. Since drinking is reinforcing for thirsty rats, approaching the tube and drinking constitutes an instrumental response that is clearly that is clearly absent with the intra-oral infusion method. However, the pairing of the taste and the subsequent aversive gastric event may also produce Pavlovian conditioning when the CS is presented by voluntary drinking.

In the voluntary drinking paradigm learned avoidance is measured by the amount of fluid consumed. There are also at least two different voluntary-drinking test methods: the one-bottle and the two-bottle procedures. In the one-bottle procedure the CS and the

control substance (almost always water) can be presented to different groups, or they can be presented to the same rats at different times, in either the same or different contexts. When different contexts are used differential conditioning to both the taste and the context may influence the rats' behaviour. With the two-bottle procedure, rats that are water deprived may quickly become satiated by drinking the available water before sampling the CS.

This analysis shows that although both I/O infusions and voluntary drinking methods produce and measure avoidance learning, the kinds of learning that occur and are measured are not always the same, and these different kinds of learning may recruit different neural structures. These considerations suggest a reason for the different effects of BLA lesions made with electrolytic and neurotoxic techniques (Dunn & Everitt, 1988; Grupp et al., 1976; Schafe et al., 1998).

Deprivation States during Training and Testing

As described above, most investigators use satiated rats with the IO infusion method of presenting the CS and deprived rats with the voluntary drinking method. When the IO method is used BLA lesions impair LTA regardless of whether or not the rats are deprived, but with voluntary presentation the absence of deprivation, either by experimental design or the use of the 2-bottle procedure, may compromise the reinforcing action of the CS and therefore eliminate the instrumental learning. The Pavlovian conditioning may or may not be affected in this situation. There are no studies examining the effect of this variable when the voluntary drinking method is used.

A Third Form of LTA Learning

The preceding section described how the LTA could be produced by Pavlovian conditioning and by instrumental learning. There is also a third possibility, that taste

avoidance could be due to a form of latent learning. Since the amygala is not involved in latent learning (Stouffer & White, 2006), electrolytic lesions of BLA might impair LTAs due to Pavlovian conditioning or instrumental learning but have no effect on an LTA based on latent learning. This could also account for some of the discrepancies in the literature on the effects of BLA lesions on LTA.

Latent Learning

Latent learning is the incidental, unreinforced and unrewarded acquisition of "neutral" information with no immediate implication for behaviour (Blodgett, 1929). The existence of the learned information becomes apparent when it later influences the acquisition or expression of some behaviour. There are two forms of latent learning (Thistlethwaite, 1951a, 1951b): One is a situation where a reinforcer is introduced into a formerly neutral environment. The second form of latent learning involves animals being pre-exposed to an incentive in a satiated state. When they are later deprived of that initial incentive they express new behaviour that allows them to obtain it. This is known as irrelevant-incentive learning.

Latent Learning with Salt Appetite

Krieckhaus and Wolf (1968) trained salt-deprived rats to press one bar for water and another bar for a salt solution followed by an extinction trial. Rats showed greater resistance to extinction on the salt than on the water bar. The authors argued that the effect was not due to enhanced reward value of the salt solution compared to the water because in a control experiment with satiated rats there were no differences in extinction rates on bars that had delivered sucrose, water or salt during training (Khavari & Eisman, 1971). Berridge and Schulkin (1989) paired either quinine or citric acid, two substances

that rats usually avoid, with salt. Later when rats were salt deprived they preferred the substance that had been paired with salt over the one that had not.

Stouffer & White (2006) distinguished between conditioned and latently learned preferences for salt using a learned cue preference paradigm. Rats were trained by exposing them to one compartment containing water and to another distinct compartment containing a salt solution that was equally preferred to water. They were then offered a choice between the two compartments with no solutions present. Rats that were salt+water deprived during training preferred their salt-paired compartments regardless of whether or not they were salt+water or water-only deprived during the test. This was interpreted as Pavlovian conditioning, in which the rats acquired a conditioned approach response to the salt-paired cues (CCP). The conditioned cues elicited the approach response during the test regardless of the rats' deprivation state.

In contrast, rats that were water-only deprived during training preferred their salt-paired compartments only if they were salt+water deprived during the test. This was interpreted as irrelevant incentive, or latent cue preference (LCP) (Thistlethwaite, 1951a, 1951b). During the training trials the salt-replete rats acquired information about the location of the salt solution but no behaviour was associated with this information. During the test the tendency to spend more time in the salt-paired compartment was due to an interaction of the latent information about salt and the deprivation state. In the CCP the presence of salt-deprivation during training resulted in the formation of a conditioned response which was elicited by the compartment cues during the test regardless of deprivation state.

These results suggest that irrelevant-incentive latent learning and conditioning are behaviourally dissociable and differ in the motivational requirements necessary for their

expression. These two forms of learning are also mediated by different neural structures. Stouffer et al (2006) showed that pre-training bilateral lesions of the lateral amygdala impaired the water and salt CCPs but not the salt LCP, reflecting the role of the amygdala in Pavlovian conditioning but not latent learning. On the other hand lesions of the entorhinal cortex impaired the salt LCP but not the CCPs. This double dissociation confirms that CCP and LCP are not only behaviourally distinct but are also mediated by different neural circuits. Lesions of the dorsal and ventral hippocampus impaired both LCP and CCP reflecting the role of the hippocampus in contextual learning (Stouffer & White, 2006)

Latent Learning in Conditioned Taste Avoidance

Much like the LCP, learned taste avoidance could also be acquired by irrelevant-incentive learning. The learned information becomes apparent when it later influences the acquisition or expression of some behaviour. If the animals are exposed to the CS in a satiated state the tastant is perceived as a neutral stimulus that is not accompanied by a biologically-relevant effect. Pairing the CS and US in this situation would lead to latent associations between the solution and the malaise. Although the rats may learn about the taste-illness association this learning may not influence behaviour until the rats are presented with the taste again. Normal rats would avoid the taste thus displaying latent learning.

This is parallel with the Stouffer and White (2006) study showing that presenting rats with a saline solution in the absence of a salt-deprived state leads to a latent salt preference when the salt information becomes relevant for survival (during salt deprivation). This form of learning is not amygdala based but rather involves the entorhinal cortex and dorsal hippocampus (Bannerman et al., 2001; Coutureau, Galani,

Gosselin, Majchrzak, & Di Scala, 1999; Coutureau, Lena, Dauge, & Di Scala, 2002; Eric M. Stouffer, 2007; Gaskin, 2007; Stouffer & White, 2006; Stouffer & White, 2007).

Multiple Memory Systems

The idea that alternate forms of learning can result in learned taste avoidance is based on the premise of multiple parallel memory systems. The concept of multiple memory systems has evolved from evidence suggesting that different types of information are stored and processed in different parts of the brain (Knowlton & Squire, 1993; McDonald, Divan, & Hong, 2004; McDonald & White, 1993; Squire, 1993; White & McDonald, 2002). This is supported by human studies of neurodegenerative diseases that impair memory as well as naturally occurring lesions resulting from surgeries or accidents (Aggleton, Shaw, & Gaff, 1992; Raked, Clemens, Walter, & Amin, 2001; Scoville & Milner, 2000).

Today the notion of several more-or-less independent neural systems that form the mammalian brain's memory system is widely accepted (Bohbot, Iaria, & Petrides, 2004; Colombo, 2004; McDonald et al., 2004; McDonald & White, 1993; McDonald & White, 1995; Packard & McGaugh, 1992; Petrides & Baddeley, 1996; Poldrack & Rodriguez, 2004). There are three main systems centred on three critical structures (Table1). This tripartite model consists of the amygdala complex that is involved in conditioned associative learning, the hippocampal system including the rhinal cortices which learns information pertaining to the relationships among different stimuli, and the dorsal striatum which learns associations between specific stimuli and motor responses to them (White & McDonald, 2002). These systems acquire information independently and simultaneously and their integrated output forms the final observed behavioural response.

Type of Learning	*What is Learned?*	*What is Expressed?*	*Central Structure*
Cognitive (S-S)	Stimulus-stimulus (S-S) associations that are made salient by their association with a reward	The S-S associations are used to guide controlled behaviors	Hippocampal formation
Habit (S-R)	Stimulus-response (S-R) associations that are strengthened through repeated pairing with a reinforcer	Responses are elicited by presentation of the stimulus, even in the absence of the reinforcer	Dorsal Striatum (Cerebellum)
Conditioning (S-Af)	A neutral stimulus (CS) is associated with a stimulus (US) that produces an affective state and an unlearned behavioral response (UR)	When the CS is encountered it elicits a behavioral response (CR) similar to the one originally elicited by the US	Amygdala

Table1. Multiple Memory Systems
The three basic types of information currently included in the multiple memory systems model.

AIMS OF THE CURRENT STUDY

The present study takes a systematic approach to studying the interaction between deprivation states and the effect of BLA lesions on the LTA. This is of interest in connection with the possibility that rats trained while satiated may acquire a latently learned taste aversion. In addition measures of consumption, USVs were recorded during all test sessions as a way of measuring the rats' affective responses to the CS. Taste avoidance has never been previously investigated in conjunction with USVs.

Based on the discussion of previous findings suggesting that motivational state determines the type of learning that occurs during the training trials the present hypothesis predicts that BLA lesions will have different effects depending on the motivational state (deprivation state) of the animals during training. Rats with a strong motivational state (produced by 23 hr water deprivation) should acquire a conditioned taste avoidance which will be impaired by amygdala lesions. Rats with a weak motivational state (3 hr water deprivation) should acquire a latent taste aversion that will not be impaired by amygdala lesions.

METHODS

Animals

Adult male Long-Evan rats (n= 209; Charles River, Saint-Constant, PQ) weighing 225 to 250 grams at the start of the experiment were housed individually and maintained on a 12/12 light/dark cycle (lights on at 7 a.m.) with food provided *ad libitum*.

Apparatus

LTA Apparatus. The test chamber was a 30 X 9 X 45 cm black Plexiglas box with a clear top. A drinking tube was suspended outside the test box with its spout protruding into the apparatus at one end, approximately 3.5 cm above the floor. The

outside of the box was surrounded by fibreglass insulation to reduce ambient background noise. A Logitech webcam was attached to the top lid of the test box and transmitted a view of the inside to a computer in a nearby room.

Ultrasonic Vocalization Apparatus.

A microphone (Knowles Acoustics FG-3329C) was fixed inside the top of the test box. The microphone was connected to a bat detector (Ultrasonic Detector D 230; Pettersson Elktronic AB; Uppsala, Sweden) which was set for frequency division mode so that the entire frequency range (10 kHz to 120 kHz) was divided by 10 and thus made audible to the human ear. The frequency division system of the D 230 retains the original input amplitude. The signal from the bat detector was amplified through a MIDI interface (US-122; Tascam TEAC Professional Division) and fed to a standard PC for recording on hard disc in .wav format using a digital recording system (MITSYN). The software recorded all bursts of sound that were above a threshold of -72 dB and ranged between 10 and 8,000 kHz (which correspond to 100 and 80,000 kHz, respectively). A programming script embedded in MITSYN converted the .wav files into text files. It analysed the files in 5 msec strings, reporting the time, amplitude, and frequency of calls. MITSYN also provided the sound information in a spectrogram format (See Figure 1 for an example).

Conditioned Freezing Apparatus.

Standard MedAssociates boxes (ENV-001, MedAssociates, Inc., Georgia, VT) with grid floors encased in sound-attenuating chambers (Model E10-10, Coulbourn Instruments, Lehigh Valley, PA) were used in this part of the experiment. A video camera hung from the middle of the ceiling of each box. All cameras were connected to a computer that used the Freezview program to record each rat's activity inside the box and analyze the freezing time.

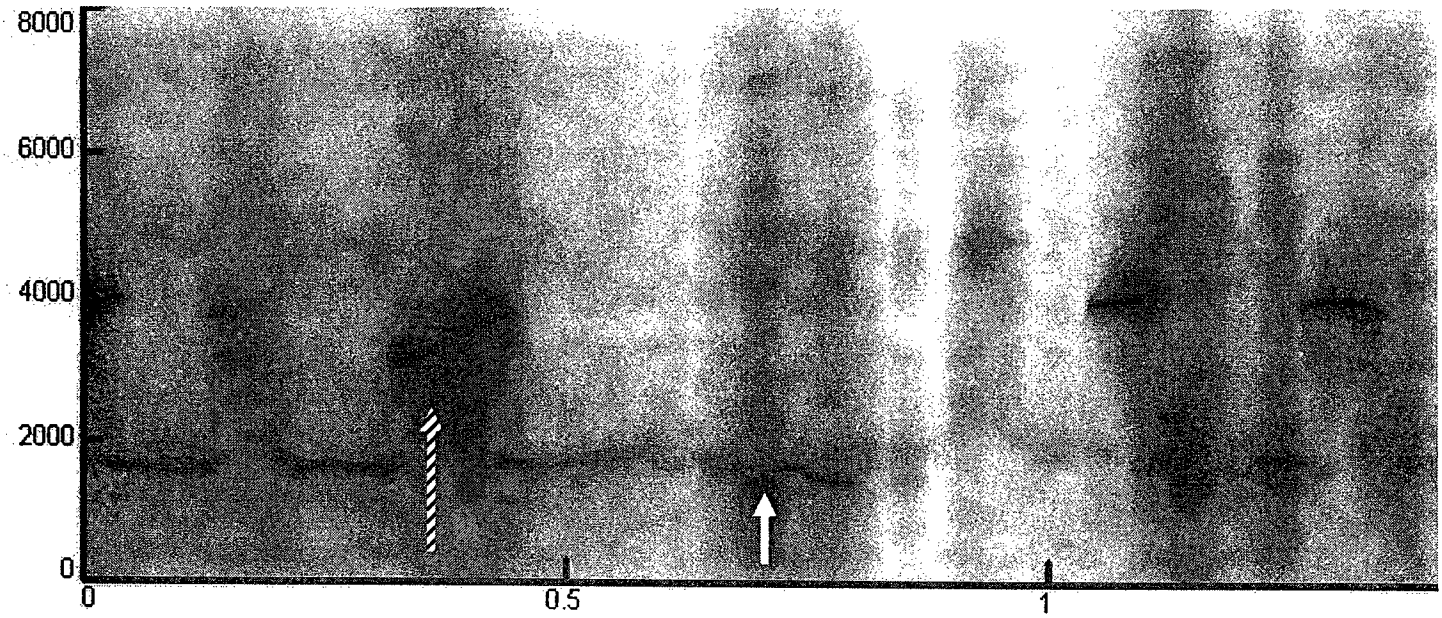


Figure 1. Spectrogram
The x-axis represents the time in seconds. In this figure 1.5 sec are depicted. The y-axis represents the frequencies in KHz/10 (or centihertz cHz). The frequencies represented were previously divided by 10 by the bat detector therefore a frequency of 2000 Hz on this spectrogram corresponds to a 20 000 Hz or 20 KHz frequency. The darker spots on the spectrogram represent calls. A striped arrow indicates an example of a high frequency call and a white arrow indicated an example of a low frequency call.

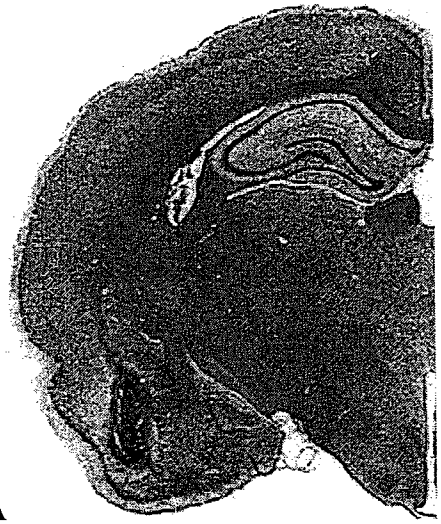
Surgery

All rats underwent stereotaxic surgery under Isoflurane anesthesia (2.5 L oxygen/min, 4 % for induction, 2.5 % for maintenance). The rats all received an intramuscular injection of the analgesic Dipyrone (0.5mg) and a subcutaneous injection of the antibiotic Tribrissen (0.5mg) at the end of the procedure. Half the subjects were given electrolytic lesions of the BLA; the other half were given sham lesions. Bilateral lesion of the BLA were made at -2.5A/P, ± 5.3 M/L and -8.2 D/V (Paxinos & Watson, 1998) using stainless steel electrodes, insulated except for a 1.0 mm tip, and a rectal ground using 1.5 mA anodal current for 20 s. Sham rats underwent the same procedure except that the electrode was aimed at coordinates 2.5A/P, ± 5.3 M/L and -7.0 D/V and no current was passed. All rats were given 5 days to recover in their home cages before the experiment started.

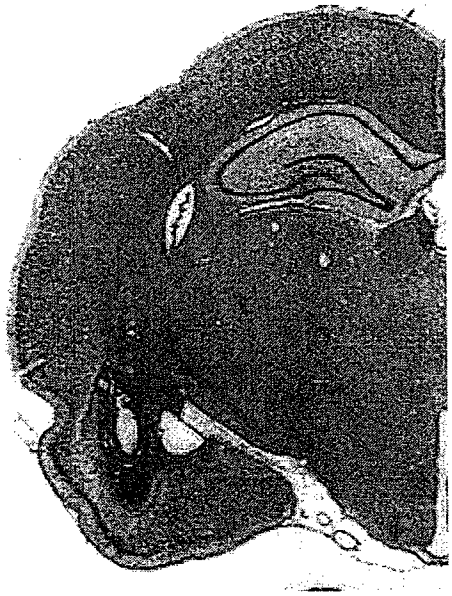
Histology

After all procedures were complete all lesioned rats were sacrificed with a lethal dose of sodium pentobarbital. Their brains were extracted, fixed and prepared for histological examination with a formol-thionin stain using standard techniques. Microscopic examination was used to validate the lesions; all the rats with lesions in locations other than in the BLA or with large lesions extending considerably beyond the BLA were excluded from this study (Figure 2)

Figure 2. Representative Lesion Placements
Photomicrographs of representative lesion placements of rats that received electrolytic BLA lesions. A, B and C represent the extent of lesions in different rats



A



B

C

Procedure

Learned Taste Avoidance

After the post-operation recovery period all rats were handled for 5 min on four consecutive days before the experiment began. On each day groups of 5-8 rats were placed in a large box and allowed to move around freely. Each rat was picked up and handled several times.

Rats were separated into 8 groups. There were 4 deprivation schedules, each made up of a combination of severe water deprivation (23hrs - D) and mild water deprivation (3 hrs – MD) applied independently to the training and test sessions (Table 2). All rats were used to their deprivation schedules during the four days of handling. Groups of rats in each deprivation condition received injections of either lithium chloride (LiCl) or saline on day 2 of the procedure.

The experimental procedure started on the day after handling. All rats were tested between 2 and 7 pm. USVs were recorded during all test trials. For rats trained in the D/D condition the water bottles were removed from the home cages at 3 pm on the previous day so that they were deprived for at least 23 hrs during all trials. On trials 1 and 3 the rats received water in the test chamber. On trials 2 and 4 they received an aspartame-based mixture made with the artificial sweetener Equal (1.3mg/ml water). On the second trial, after the rats drank the sweet solution for the first time, they were injected intraperitonneally (i.p.) with LiCl (0.15 M – 25mg/kg) or saline (1ml/kg) immediately after being removed from the test chamber. No injections were given on any other day. Taste avoidance was tested on day 4 when the sweet solution was offered for the second time. The amount drunk on each day was recorded.

Table 2. Distribution of the Groups
Rats were divided into 4 groups following the combination of training and testing deprivation schedules. Deprived rats (D) and were only allowed to drink water for one hour each day in their home cage. Mildly deprived (MD) rats were allowed to drink for most of the day except for the 3 hours preceding training or testing.

Groups	Training	Testing	n
D/D	23hrs deprivation	23hrs deprivation	42
D/MD	23hrs deprivation	3hrs deprivation	48
MD/MD	3hrs deprivation	3hrs deprivation	47
MD/D	3hrs deprivation	23hrs deprivation	46

In the MD/MD condition the training protocol was the same as in the D/D condition except that the water bottles were removed from the home cages 3 hours prior to each training trial. Since rats normally drink very little during this part of their daily light cycle this produced a very mild deprivation state. The alternation of water and Equal solution and the injection schedule was the same as in the D/D condition.

In the MD/D condition the rats were deprived for 3 hours for each of the first 3 trials, and for 23 hr prior to the final trial. The rats in the D/MD condition were deprived for 23 hr prior to each of the first 3 trials and for 3 hr prior to the 4^{th} trial. Alternation of water and the Equal solution was the same as described above.

A preliminary consumption test was done to determine the concentration of Equal to use in the experiments. The rats were given 30 ml of sucrose (10%), aspartame (Equal) or sucralose (Splenda) solutions in their home cages for 1 hour on consecutive days. The rats consistently failed to drink the sucralose solution. The concentration of the aspartame solution was adjusted until the rats drank the same amount as the sucrose solution. They drank equal amounts of 10% sucrose and the 1.3mg/ml Equal solution.

Conditioned Freezing

After the LTA experiment all rats were given water *ad lib.* for a few days and then tested in a conditioned freezing task. Rats were pre-exposed to the shock apparatus for 10 minutes on the first day. On day 2 the rats were put back in the shock apparatus where they received 5 consecutive electrical shocks of 1 mA intensity and 1.0 sec. duration at 1 min intervals. They were put back in the same apparatus on the following day for another 10 min period. No shocks were administered and amount of time spent freezing was measured.

USV Analysis

The text file produced by MITSYN was filtered with an Excel template which further segregated the calls based on frequency and duration criteria. A low-frequency call was defined as a burst ranging between 1500 and 2500 kHz (actual frequency divided by 10) with a minimum duration of 100 msec, while a high-frequency call was defined as a burst above 3500 kHz with a minimum duration of 35 msec. Sample results of this analysis were compared by listening to a number of sessions and counting the high- and low-frequency calls. This exercise confirmed that the calls counts identified by the Mitsyn program and subsequent analysis were accurate.

The raw frequencies of high and low calls were combined by calculating a ratio: high calls/(high calls + low calls). The value of this ratio varies between 0 and 1. A score of 0 would be obtained if only low frequency calls were produced, and would be interpreted as indicating a state of strong negative affect. A score of 1 would mean that only high frequency calls were made, and would be interpreted as an indication of very strong positive affect. Since there are very large individual differences in rates of vocalization among rats (Burgdorf, Panksepp, Brudzynski, Kroes, & Moskal, 2005) the call ratio had the effect of normalizing these differences. Another useful characteristic of this ratio is that it integrates two measure of affect that are thought to represent different points on the same scale (Young, 1959; 1962), providing a single measure reflecting a rat's momentary affective state.

As is standard with ratio data (Takane & Fergusson, 1992) the call ratios were treated with an arcsin square root transformation ($P = arcsin \sqrt{(ratio)}$. Analyses of

variance (ANOVAs) were used to compare the vocalizations of each group. Fisher's LSD was used as a post-hoc test when a significant difference was detected by the ANOVAs.

RESULTS

D/D condition:

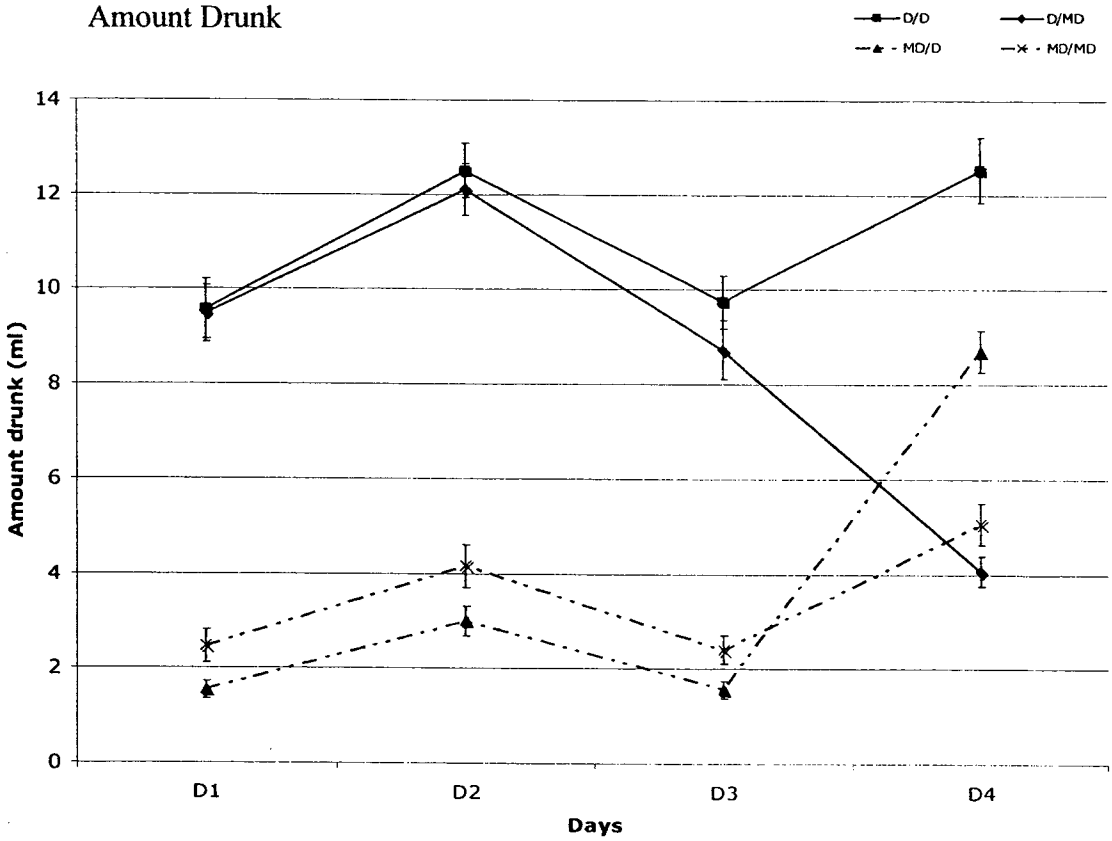
Figure 3a depicts the amounts of liquid drunk. A repeated measures ANOVA followed by Fisher's LSD post hoc test revealed a significant effect of drug x lesion x day [$F(3, 145) = 3.661$, $p<0.05$] showing that fluid consumption varied among the groups across days. There were no significant differences in consumption among the four groups on D1 ($p >0.05$). All groups consumed significantly more liquid on D2 than on D1 ($p < 0.01$) indicating that the Equal solution was more palatable than water. Despite our best attempt at randomization the BLA-LiCl group drank significantly more than the Sham-Sal group ($p <0.05$) on D2, while consumption for all other groups was similar. Fluid consumption decreased on D3 (water) in comparison with D2 (Equal) for all groups ($p<0.05$). The reduced consumption of the Sham-LiCl group in comparison to that of the Sham-Sal group on D4 (Equal) is clear, demonstrating the acquisition of the LTA. In contrast, the consumption of the BLA-LiCl group was normal, indicating that the lesion completely eliminated the LTA. The statistical analysis of the D4 data is presented below.

Figure 3b shows the USV ratios for this condition. A repeated measures ANOVA shows an effect of lesion x drug across days [$F(3,154) = 0.022$, $p<0.05$]. Fisher's LSD post-hoc tests show that the vocalization ratios observed on D2 were all significantly higher than on D1 ($p < 0.05$) suggesting that all groups showed a similar increase in positive affect on D2 compared to D1. We can thus conclude that the Equal solution is

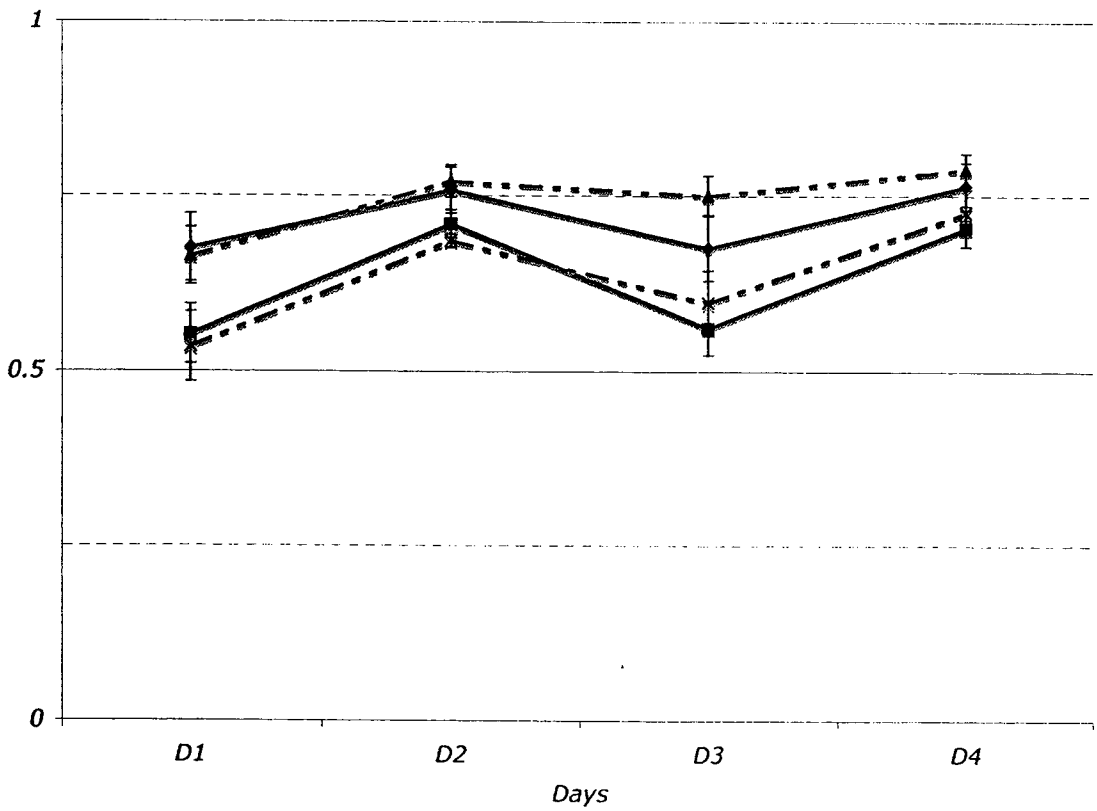
Figure 3 a) The rats in the same deprivation condition during training (D1-3) behaved similarly until the test day (D4). The drinking patterns of the rats diverged on D4 since their deprivation state was altered. Rats that were deprived for 23 hrs prior to testing drank significantly more than rats that were deprived only 3 hrs before testing. **b)** There is no notable significant difference between the USV calls emitted by the Saline rats regardless of the type of lesion they received.

Figure 3

A Amount Drunk



B USV Ratios



more palatable (rewarding) than water since the majority of animals drank more of it and produced higher calls ratios. The ratios of D3 show a slight decrease for all groups compared to D2 ($p < 0.05$). This corresponds to the decrease in consumption on D3. The ratios on D4 also correspond to the consumption data, in that the ratio for the Sham-LiCl group is lower than the ratios for the other 3 groups.

Similar patterns of consumption and call ratios over days 1 – 3 were observed in all four deprivation conditions, although the amounts consumed by rats in the MD groups were lower than those in the D groups. In all groups the rats drank more of the sweetened solution and had higher call ratios on D2 (Equal) compared to Day 1 (water), confirming that prior to pairing with LiCl the Equal solution was preferred to water, and that consumption of this preferred solution was accompanied by positive affect. Accordingly, these data are not shown – and the analysis of the results focuses on the learned taste aversion as measured by consumption and call ratios on D4.

Fluid Consumption

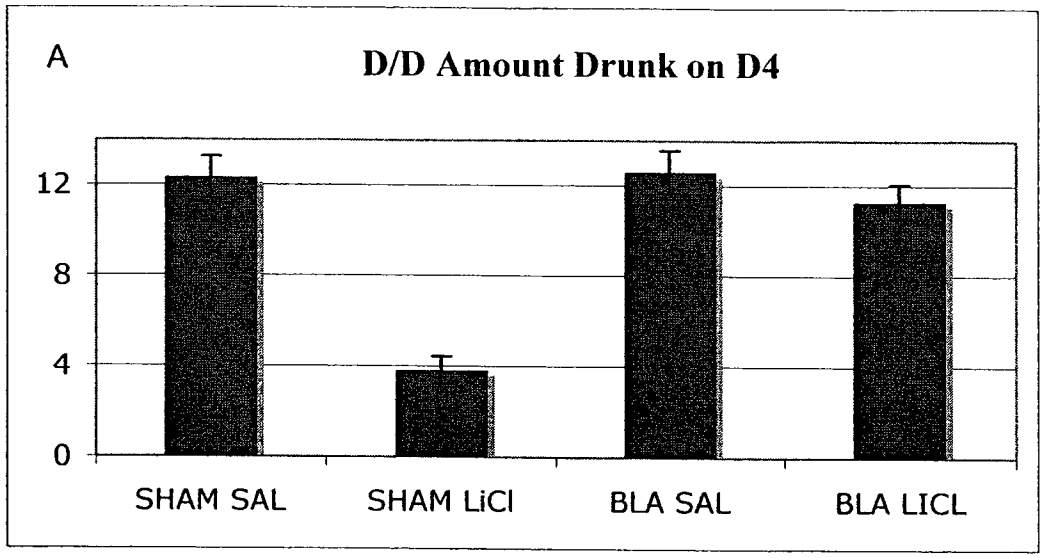
A one-way ANOVA on the consumption data for D4 (Figure 4a) shows that the Sham-LiCl group drank significantly less sweet solution than the BLA-LiCl [$F(1,38) = 15.68$, $p < 0.001$] and the saline groups (BLA-Sal $p < 0.001$; Sham-Sal $p < 0.001$). Rats in the BLA-LiCl, BLA-Sal and Sham-Sal groups all drank statistically equal amounts. This indicates that the Sham-LiCl group acquired a conditioned avoidance whereas the other groups did not. The BLA lesion blocked the taste avoidance in this condition.

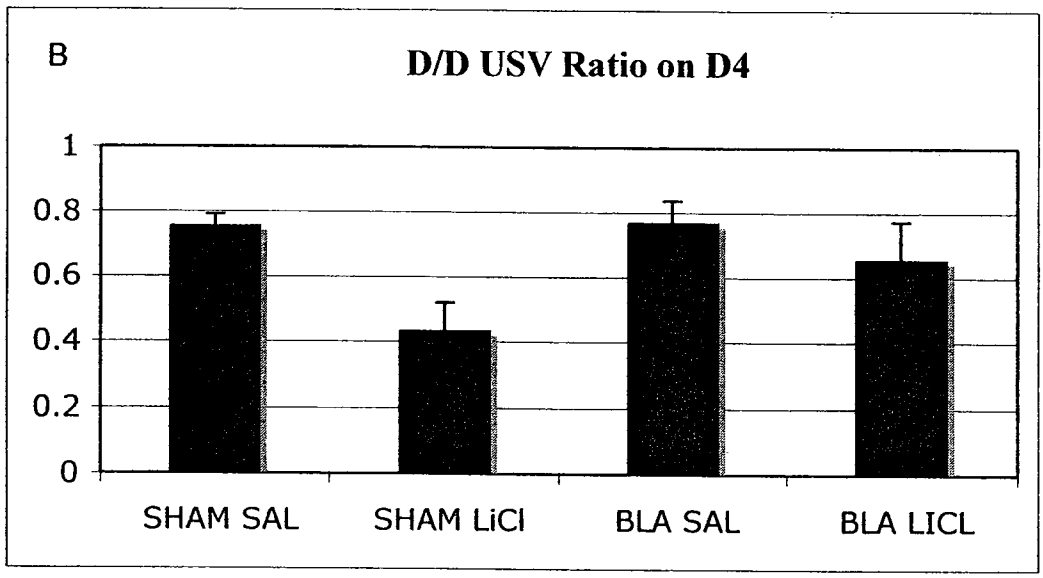
Vocalizations

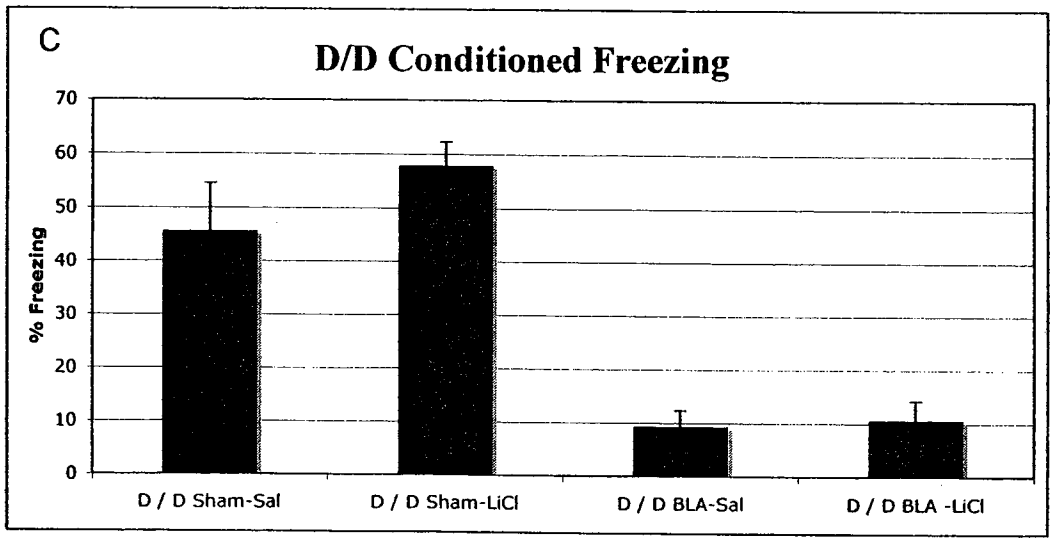
Figure 4 b shows the vocalization ratios on D4. The USV ratio analysis parallels the drinking analysis [$F(1,38) = 6.29$, $p < 0.05$)]. The Bla-LiCl, Bla-Sal and Sham-Sal

Figure 4. D/D Results
A) Amounts drunk on D4 for the D/D condition. Sham-LiCl group drank significantly less than the other groups. **B)** Call rations on D4 for the D/D condition. The calls reflect the drinking behaviour whereby only the Sham-LiCl show a negative ratio below 0.5. **C)** % Freezing in conditioned freezing task. The sham groups consistently displayed more freezing behaviour than the BLA lesioned groups.

Figure 4


A
D/D Amount Drunk on D4
12
8
4
0
SHAM SAL
SHAM LiCl
BLA SAL
BLA LICL


B
D/D USV Ratio on D4
1
0.8
0.6
0.4
0.2
0
SHAM SAL
SHAM LiCl
BLA SAL
BLA LICL


C
D/D Conditioned Freezing
% Freezing
70
60
50
40
30
20
10
0
D / D Sham-Sal
D / D Sham-LiCl
D / D BLA-Sal
D / D BLA -LiCl

groups all show significantly higher call ratios than Sham-LiCl ($p < 0.05$; $p < 0.05$ and $p < 0.01$ respectively). The Bla-LiCl group was no different than the two saline groups ($p > 0.05$) (Figure 4b). These findings correspond to those for consumption: decreased consumption, presumably due to an LTA is accompanied by decreased call ratios, suggesting the presence of negative affect.

Conditioned Freezing

The conditioned freezing data for this condition are shown in Figure 4c. A one way ANOVA shows a significant difference among the groups [$F(3,145) = 4.32$, $p<0.05$]. All the BLA lesioned groups were impaired on this measure regardless of the treatment they received during the taste avoidance learning. The BLA-LiCl and BLA-Sal groups froze significantly less than the two sham groups ($p < 0.001$).

Conclusion

On D4, when offered the paired solution, both consumption and call ratios for the Sham-LiCl group decreased significantly compared to the other groups, consistent with the hypothesis that the reduced consumption was accompanied by negative affect. The BLA lesions apparently prevented the formation of the taste avoidance association and also appeared to eliminate the negative affect, as suggested by the high call ratios.

It is unclear from these data, if the conditioning process involved both drinking and negative affect, or if only one of these behaviours was learned as a result of the Equal – LiCl pairing. If reduced consumption but not negative affect was learned, it could have caused the negative affect. Conversely, if negative affect but not reduced consumption was learned, it could have caused the reduced consumption.

D/MD condition:

Fluid Consumption

The data for amount drunk on D4 in this condition are shown in Figure 5a. There was a significant difference among the groups [F (1,42) = 54.964, $p<0.05$]. The two groups that received LiCl injections drank significantly less that the groups that received Saline injections (both $ps<0.05$) and the difference in the amounts drunk by the BLA-Sal and Sham-Sal group drank were not significantly different ($p >0.05$). On the other hand the BLA-LiCl group drank significantly more than the Sham-LiCl group ($p<0.05$), which can be interpreted as an attenuation of the learned taste aversion.

Vocalizations

The call ratio data for D4 are shown in Figure 5b. Only the main effect of LiCl vs Saline was significant [F (1,42) = 9.1388, $p<0.05$]. The ratio for the Sham-LiCl group was significantly lower than the ratio for the both the saline groups ($p < 0.05$) and for BLA-LiCl group ($p < 0.05$). The differences between the ratio of the Bla-LiCl group and either the BLA-Sal ($p = 0.102$) or Sham-Sal ($p = 0.068$) groups were not significant.

Conditioned freezing

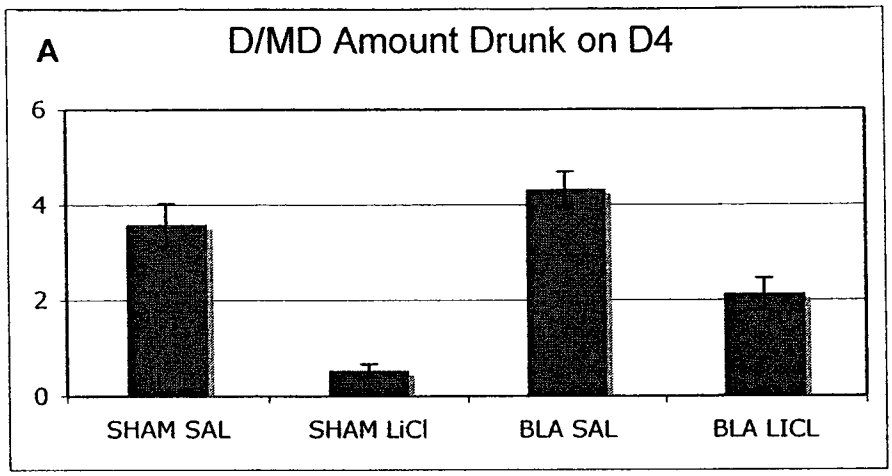
The conditioned freezing data are shown in Figure 5b. There was a significant difference among the groups [F (3, 167) = 7.345, $p < 0.001$]. The BLA lesions impaired conditioned freezing completely compared to the sham groups ($p<0.001$).

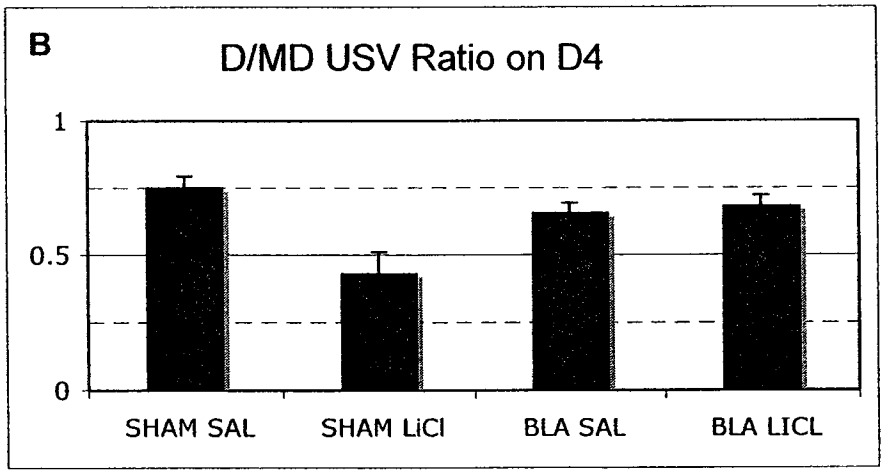
Conclusion

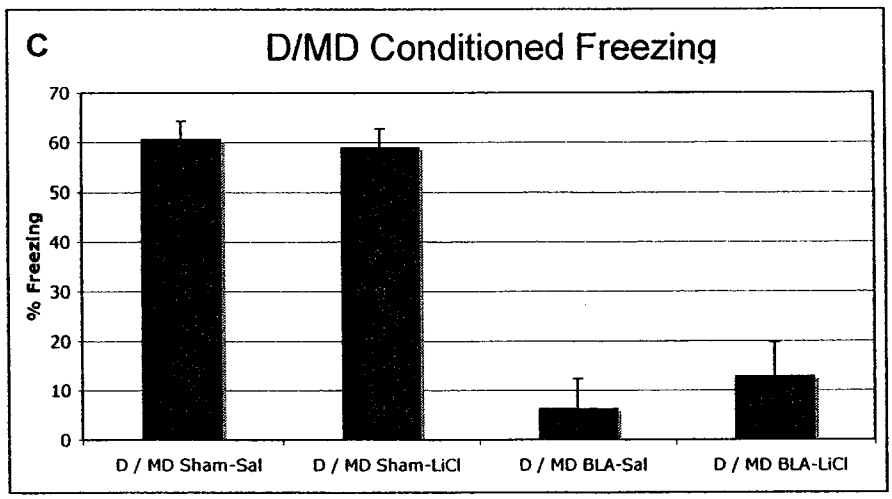
These results suggest that the BLA lesion abolished the expression of the conditioned negative affect, yet this was not sufficient to completely abolish the taste avoidance behaviour. Another mechanism independent of both the BLA and negative affect may be contributing to the expression of conditioned taste avoidance in this

Figure 5. D/MD Results
A) The BLA lesion only attenuated the taste avoidance but did not reduce it to a level similar to sham animals. **B)** The BLA lesion abolished the negative affect usually expressed with taste avoidance. **C)** the BLA lesions impaired the conditioned freezing.

Figure 5


A
D/MD Amount Drunk on D4
6
4
2
0
SHAM SAL
SHAM LiCl
BLA SAL
BLA LICL


B
D/MD USV Ratio on D4
1
0.5
0
SHAM SAL
SHAM LiCl
BLA SAL
BLA LICL


C
D/MD Conditioned Freezing
% Freezing
70
60
50
40
30
20
10
0
D / MD Sham-Sal
D / MD Sham-LiCl
D / MD BLA-Sal
D / MD BLA-LiCl

situation. This will be addressed in the discussion section. The results also suggest that negative affect and reduced consumption do not always vary in parallel and, in particular that negative affect is not required for reduced consumption. Notwithstanding these varied effects, the lesions were completely effective at impairing the conditioned freezing task, suggesting that they were effective.

MD/MD condition

Fluid consumption

The data for this condition are shown in Figure 6a. There was a drug effect in this condition [$F(1,31) = 8.00$, $p<0.001$]. The two groups of rats injected with LiCl on D2 drank significantly less on D4 than the groups injected with saline (BLA-LiCl vs Bla-Sal, $p<0.05$; Sham-LiCl vs Sham-Sal. $p<0.001$ for Sham-Sal). The BLA-LiCl and Sham-LiCl did not differ ($p = 0.902$).

Vocalizations

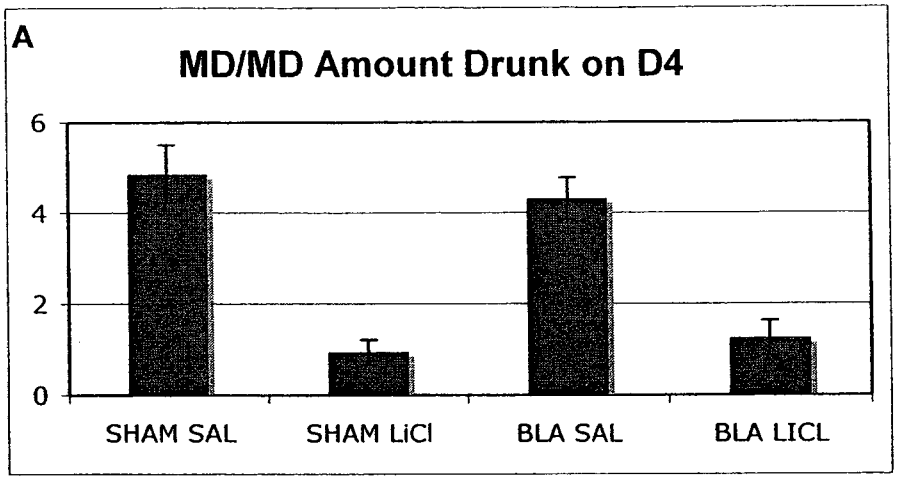
The data for the vocalizations are shown in Figure 6b. There was a significant difference among the groups [$F(1,31) = 8.96$, $p<0.001$]. The call ratios for the rats injected with LiCl were lower than those of the saline-injected rats in both the lesioned and sham-lesioned rats (BLA-LiCl: $p < 0.001$ vs BLA-Sal, $p < 0.001$ vs Sham-Sal; Sham-LiCl: $p < 0.005$ vs Bla-Sal, $p < 0.001$ vs Sham-Sal). There were no differences between the two saline groups ($p = 0.969$) or between the two LiCl groups ($p = 0.503$) (Figure 5b).

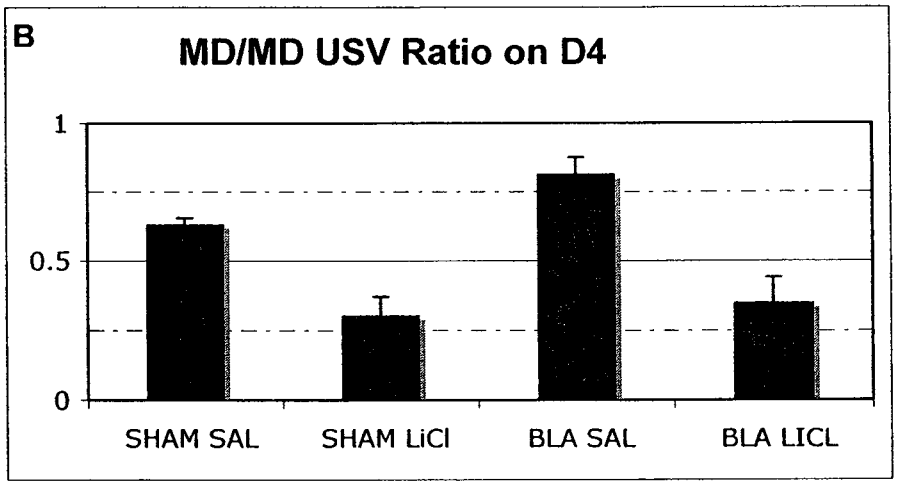
Conditioned freezing

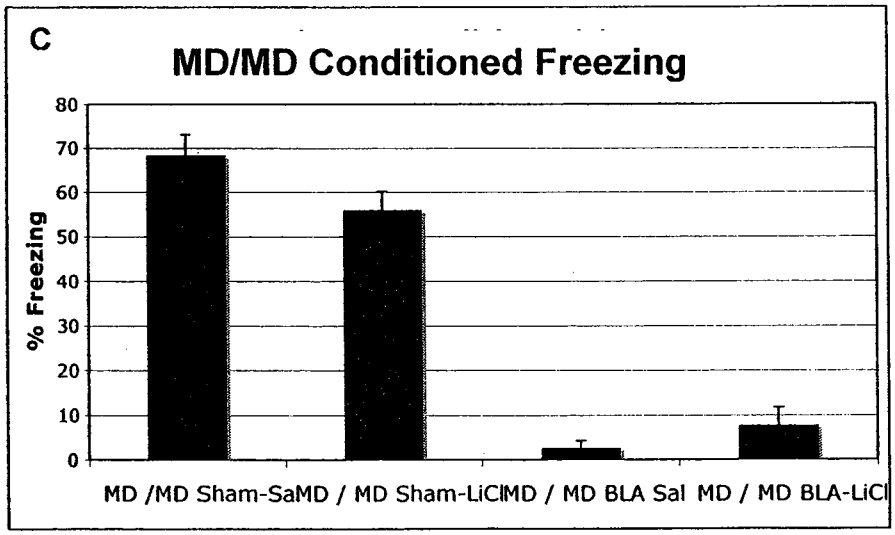
These data are shown in Figure 6c. There was a significant difference among the groups [$F(3, 142) = 31.863$; $p < 0.001$]. The lesioned rats froze significantly less than the sham-lesioned rats regardless of which drug was administered during the learned taste avoidance ($p<0.001$).

Figure 6. MD/MD Results
A) The BLA-LiCl group drank as little as the Sham-LiCl group and significantly less than the saline groups. **B)** The BLA-LiCl and Sham-LiCl groups both expressed a negative affect as inferred by a low ratio. **C)** The BLA lesions impaired the conditioned freezing task.

Figure 6


A
MD/MD Amount Drunk on D4
6
4
2
0
SHAM SAL
SHAM LiCl
BLA SAL
BLA LICL


B
MD/MD USV Ratio on D4
1
0.5
0
SHAM SAL
SHAM LiCl
BLA SAL
BLA LICL


C
MD/MD Conditioned Freezing
% Freezing
80
70
60
50
40
30
20
10
0
MD /MD Sham-Sa
MD / MD Sham-LiCl
MD / MD BLA Sal
MD / MD BLA-LiCl

Conclusion:

The rats in the MD/MD condition that received LiCl injections exhibited both taste avoidance and negative affect on the test trial compared to the rats that received saline injections; this was equally true for both amygdala and sham-lesioned rats. On the conditioned freezing test, however, the amygdala lesioned rats froze significantly less than the sham lesioned rats. This evidence that the lesions had a functional effect, together with the fact that there were no obvious differences in lesion size and location across the groups in which these measures were and were not affected, suggests that that, in this deprivation condition, neither the taste avoidance nor the aversive calls is the result of an amygdala-mediated conditioned response.

Since the rats were only very mildly deprived during training on D2, it is possible that they did not acquire conditioned responses, but instead learned about the relationship of the taste of the Equal and the aversive effects of the LiCl injection in a latent manner, as was previously shown to be the case for learning about the location of salt (Stouffer & White, 2007). This possibility will be addressed in the General Discussion.

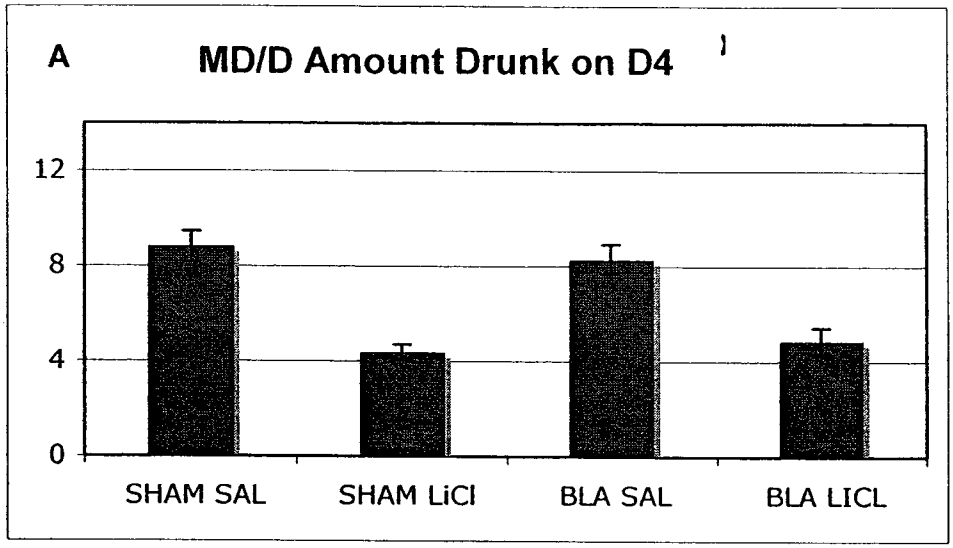
MD/D condition

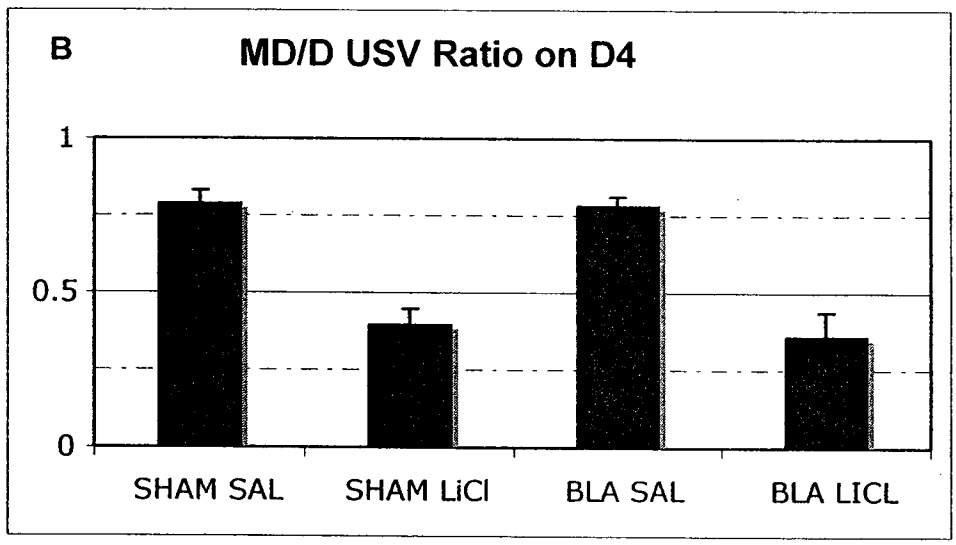
Fluid consumption

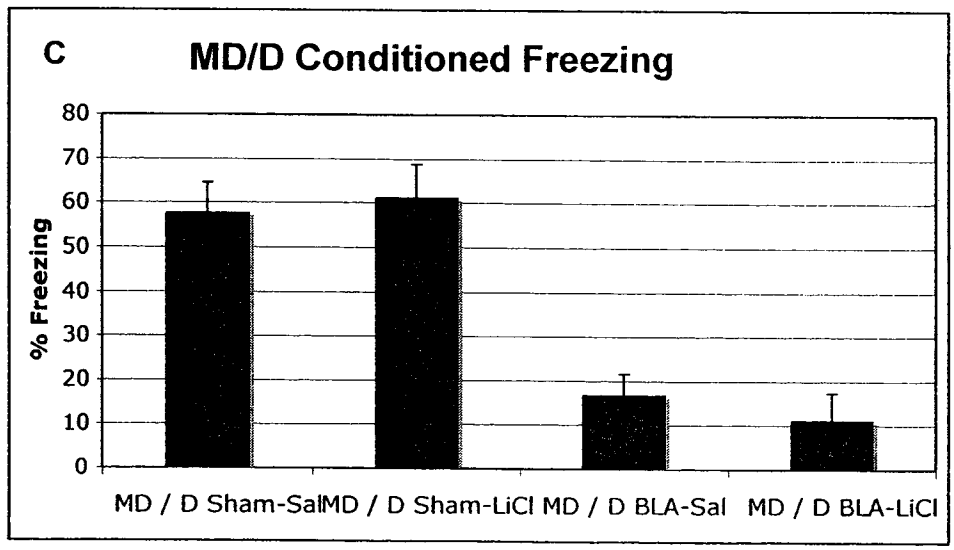
The consumption data for the MD/D condition are shown in Figure 7a. There was a significant effect of drug x day [$F(1,43) = 50.01$, $p<0.01$], but no main effect of lesion ($p > 0.05$). The groups injected with LiCl drank significantly less than the groups injected with Saline (BLA-LiCl: $p < 0.001$ for BLA-Sal and $p < 0.001$ for Sham-Sal; Sham-LiCl: $p < 0.001$ for BLA-Sal and $p < 0.001$ for Sham-Sal). There were no significant differences between the means for the saline ($p < 0.05$) or the LiCl ($p<0.05$) groups.

Figure 7. MD/D Results
A) As in the MD/MD condition the amygdala lesion did not have an effect on taste avoidance learning as the BLA-LiCl group drank as little of the sweetened solution as the Sham-LiCl group.**B)** The USV calls also follow the same trend. The BLA-LiCl group displays a negative ratio just as the Sham-LiCl group does. **C)** The Lesions attenuated the conditioned freezing

Figure 7


A
MD/D Amount Drunk on D4
12
8
4
0
SHAM SAL
SHAM LiCl
BLA SAL
BLA LICL


B
MD/D USV Ratio on D4
1
0.5
0
SHAM SAL
SHAM LiCl
BLA SAL
BLA LICL


C
MD/D Conditioned Freezing
% Freezing
80
70
60
50
40
30
20
10
0
MD / D Sham-Sal
MD / D Sham-LiCl
MD / D BLA-Sal
MD / D BLA-LiCl

Vocalizations

The vocalization data are shown in Figure 7b. There is a significant drug x day effect [$F(1,43) = 65.35$, $p<0.001$]. There were no significant differences between the BLA-LiCl and Sham-LiCl groups ($p < 0.5$) or between the BLA-Sal and Sham-Sal groups ($p < 0.5$). The means for the LiCl groups were significantly different from the saline groups (BLA-LiCl: $p < 0.001$ for BLA-Sal and $p < 0.001$ for Sham-Sal; Sham-LiCl: $p < 0.001$ for BLA-Sal and $p < 0.001$ for Sham-Sal), showing that BLA lesions did not affect the acquisition and/or expression of conditioned affect in this condition.

Conditioned Freezing

These data are shown in Figure 7c. There was a significant effect of lesion [$F(3,174) = 12.334$, $p <0.001$] and the lesions significantly impaired conditioned freezing ($p < 0.05$) (figure 6c).

Conclusion

The rats in the MD/D condition acquired and expressed both taste avoidance and negative affect, which were expressed even though the rats were not deprived, and amygdala lesions had no effect on either behavioural measure. The similarity between these effects and those in the MD/MD condition shows that training in the MD condition results in learning that does not involve the amygdala regardless of whether or not the rats are deprived during the test.

GENERAL DISCUSSION

Normal rats exhibited learned taste avoidance (LTA) accompanied by reductions in the call ratios indicative of an aversive affective state regardless of whether they were trained under strong or mild deprivation states. The effects of lesions of the basolateral and lateral nuclei of the amygdala (BLA) on these two variables are summarized in Table

3. The lesions eliminated the LTA and the call ratio reductions when there was a strong motivational state during both the conditioning and testing phases (D/D). When the motivational state changed from strong during conditioning to mild during testing (D/MD) the BLA lesions attenuated the LTA but completely eliminated the call ratio reductions.

In contrast, when conditioning occurred under a mild motivational state amygdala lesions had no effect on either the LTA or the reduced call ratios (MD/MD and MD/D). This suggests that the LTA can be acquired in at least two different ways, amygdala-dependent and non-amygdala-dependent, and that the way in which it is learned is determined by the rats' motivational, or deprivation state. This finding is consistent with the hypothesis presented in the introduction, that LTA acquired in a non-deprived state would not be affected by amygdala lesions, possibly because it is due to some form of latent learning that does not depend on this structure.

Amygdala lesions did not affect the call ratios in rats trained under mild motivational states (MD/MD, MD/D), but did affect them in the rats trained under the strong motivational state. This indicates that although the lesions may or may not impair the acquisition and/or expression of learned affect, they do not affect the ability of the rats to emit the calls that produce the ratios used to measure affect.

Structures such as the amygdala, the entorhinal cortex, the hippocampus and sensory cortical regions (Insausti et al., 1997; Burwell and Amaral, 1998a; 1998b; Witter et al., 2000; Sewards and Sewards, 2003) are thought to be involved in brain systems that represent and store affect-related information (see discussion below). Outputs from these

	Drink	Call R
D/D	↑	↑
D/MD	↑	↑
MD/MD	—	—
MD/D	—	—

Table 3: Effects of Amygdala lesions on LTA and accompanying call ratios
Pairing with LiCl reduced both consumption and call ratios in Control (Sham lesioned) rats; arrows show the effects of lesions on these two variables. In the Drink column up-arrows mean that consumption increased compared to controls, indicating that the lesion eliminated or attenuated the LTA. In the Call R column up-arrows mean that the lesion increased the call ratios compared to controls, indicating that the lesions rats expressed positive rather than negative affect. Horizontal lines mean the lesions had no effect on the taste aversion.

systems control various affect-related functions, including approach and withdrawal behaviour, positive and negative affect, and the calls used to calculate the ratios in the present experiment. With respect to the calls, Burgdorf, Wood, Kroes, Moskal and Panksepp (2007) found that electrical stimulation of the prefrontal cortex, nucleus accumbens, ventral pallidum, lateral hypothalamus, ventral tegmental area and raphe of rats all produced increases in high frequency (50 kHZ range) calls. (No study has been published to date concerning the neural substrates supporting 20KHz call production.) Some or all of these structures may be involved in producing calls that are related to both earned and unlearned affect.

The present study is the first to investigate the fluctuations of rat USVs during learned taste avoidance and is the only behavioural study to date to record both low and high USVs simultaneously. It is also the first study of taste avoidance to use a mild deprivation state during training with the CS presented by voluntary drinking instead of oral infusion. One consequence of these innovations is that direct comparisons and analogies with the results of other experiments in the literature are often not possible.

Since all BLA lesions in this study were made prior to any experimental manipulation it was not possible to determine whether the lesion effects were due to impairments of acquisition or expression of the learned behaviours studied. Future investigations could examine contribution of the BLA to these stages of learning using temporary inactivation techniques.

D/D Condition:

In the D/D condition the animals were water deprived for 23 hours during training and testing; the lesions completely eliminated the LTA, and the accompanying reduction in the call ratios. As described in the introduction, most previous studies have found that

electrolytic lesions of the amygdala that include the BLA attenuate the LTA (Dunn and Everitt, 1988; Grupp, et al., 1976; Aggleton, et al., 1986; Bermudez-Rattoni, 1986), although larger lesions have been reported to abolish it completely (Bermdez-Rattoni, et al., 1986), as in the present study. In addition to lesion size, another possible reason for the difference in the degree of elimination may be the size of the test cage. A small cage such as the one used in the present study (270 cm^2), would restrict a rat's normal exploratory movements, possibly reducing the instrumental component of the learned aversion and increasing the contribution of the conditioned aversion, making it more susceptible to BLA lesions. By comparison, in studies that found attenuation rather than complete elimination of the LTA, the test cage used by Aggleton et al measured 900 cm^2 and by Bermudez-Rattoni, 460 cm^2.

Based on previous reports of the effects of amygdala lesions (discussed in the Introduction), the elimination of both the LTA and reduced call ratios in the D/D condition by the lesions made in the present study was likely due to disruption of the neural basis of Pavlovian conditioning. The amygdala is thought to be central for the formation of stimulus-affect (S-Af) associations in which a neutral stimulus (CS) is paired with an unconditioned stimulus (US) that, without any prior learning, produces a UR consisting of an approach or avoidance response (directly observable) and positive or negative affect (which can be inferred from the call ratio) (Gilbert et al., 2003; Gilbert & Kesner, 2002; McDonald et al., 2004; McDonald & White, 1993; Ono, Nishijo, & Uwano, 1995; Packard & Wingard, 2004).

The implication of this interpretation is that the normal rats, having experienced pairing of a CS (the taste of the solution) and a UR (gastric malaise produced by the LiCl injection) acquired a conditioned aversive gastric response to the taste of the solution

during the training trials, and that contact with the CS during the test trial resulted in the experience of conditioned gastric malaise which was reflected in the reduced call ratios and suppressed drinking. Accordingly, the LTA acquired in the presence of a strong motivational state is a *conditioned* taste aversion (CTA). This interpretation of the amygdala-dependent CTA has been confirmed by a number of studies (Reilly & Bornovalova, 2005; St.Andre & Reilly, 2007).

The present finding that BLA lesions eliminate the CTA is consistent with reports of other experiments that used rats deprived for at least 20 hrs, electrolytic lesions that affected the basolateral nucleus, and presentation of the CS by voluntary drinking for the first time during pairing and alone during the test exhibit either complete or partial elimination of the behavior (Aggleton et al., 1981; Bermudez-Rattoni, et al., 1986: Grupp, et al., 1976; Dunn and Everitt, 1988). However, variations in any part of this procedure have resulted in failures of amygdala lesions to impair the CTA. The use of neurotoxins (ibotenic acid or NMDA) to make the lesions (F. Bermudez-Rattoni & McGaugh, 1991; Dunn & Everitt, 1988; Hatfield, Han, Conley, Gallagher, & Holland, 1996; Schafe et al., 1998; Yamamoto & Fujimoto, 1991) (Dunn & Everitt, 1988; Schaffe, et al., 1998; Yamamoto & Fuji, 1991; Bermudez-Rattoni, et al., 1991; Gutierez, et al., 1999; Hatfield, et al., 1992), pre-exposure to the CS (Bermudez, et al., 1991; Dunn & Everitt, 1988; St-Andre & Reilly, 2007), or the use of the 2-bottle test in which drinking water presented as the alternative to the CS quickly "undeprives" the rats, making them equivalent to rats in the present mild deprivation condition (Aja, Sisouvong, Barrett, & Gietzen, 2000; Freeman, Rice, & Riley, 2005; Galaverna et al., 1993; Schafe et al., 1998), have all been reported to eliminate the effect of lesions that involve the basolateral amygdala on the LTA. It has also been reported that electrolytic lesions of the

amygdala that do not affect the BLA but respect the above conditions have no effect on the LTA (Aggleton et al., 1981; Bermudez-Rattoni et al., 1986).

Although neurotoxic lesions generally do not impair (or only partly impair) the LTA in deprived rats when the CS is presented by allowing the rats to drink voluntarily, these lesions do impair the LTA when the CS is presented by intraoral (IO) infusion, regardless of whether the test is also done with IO infusion, or by drinking. (Schaffe et al., 1998). These authors argued that this effect is due to the absence of instrumental learning with IO presentation of the CS, resulting in a LTA that is entirely due to Pavlovian conditioning. Since the I/O method isolates the Pavlovian component of the behaviour, its impairment by BLA lesions is consistent with the importance of this brain area for Pavlovian learning (Antoniadis & McDonald, 2000, 2001; Balleine & Killcross, 2006; Everitt, et al., 2003; Fanselow & Gale, 2003; Ferreira, Ferry, Meurisse, & LÇvy, 2006; Gallagher, Graham, & Holland, 1990; Holahan & White, 2004; McDonald & White, 1993). Although Pavlovian conditioning accounts for the amygdala-dependent LTA, the fact that neurotoxic lesions have no effect when the CS is presented by drinking suggests that the LTA is also learned in some other, amygdala-independent way.

Wilkins & Bernstein (2006) found differences in expression of Fos-like immunoreactivity (FLI) in the BLA , CeN and IC depending on the conditioning method used. When rats were trained with IO infusion high levels of FLI were expressed in the CeN but not in the BLA, the IC or the nucleus of the solitary tract. In contrast, training with voluntary drinking using one-bottle resulted in increased cellular activation in BLA, CeN, IC and nucleus of the solitary tract. These findings are consistent with the idea that the LTA is learned in different ways by different brain systems and, specifically, with the failure to impair LTA of lesions confined to the CeN when the CS is presented by

drinking (Yamamoto & Fuji, 1991; Sakai & Yamamoto, 1999; Hatfield, 1992; Bermudez-Rattoni, 1986; Aggleton, et al., 1981).

In general, the findings discussed show that the effects of amygdala lesions on the LTA are determined by a complex interaction among lesion method, lesion location within the amygdala, deprivation state, conditioning method, and other factors.

D/MD Condition

In the D/MD condition the present BLA lesions partly blocked the LTA. In contrast, the negative call ratio was completely eliminated by these lesions, suggesting that they may have eliminated a conditioned aversive response to the CS, acquired by the pairing of saccharin and the effect of LiCl. This finding, together with the fact that the LTA was attenuated but not eliminated by the BLA lesions indicates that some form of learning other than Pavlovian conditioning involving a different neural system may have occurred in parallel with the Pavlovian conditioning.

Instrumental Processes

Instrumental learning can take several forms, all of which involve learning about the association between a rewarding or aversive event some aspect of the situation or environment in which it occurs. Two such processes are discussed here, one focused on acquisition, the other on retrieval and expression.

Contextual Association.

In this form of learning the aversive state produced by LiCl can become associated with the environmental context in which it occurs. This would result in avoidance of the location of the drinking tube and, consequently, in reduced drinking. The idea that independent associations are formed between affective states and tastes or places is well known in the drug literature. For example, morphine has both rewarding

and aversive effects (Bechara, Pridgar, Gerard, & van der Kooy, 1993; Bechara, Zito, & van der Kooy, 1987; Bozarth & Wise, 1983; Mucha & Herz, 1985; Olmstead & Franklin, 1997; Parker, 1991; Wise, Yokel, & DeWit, 1976). In one demonstration of how these are independently associated, White et al (1977) trained rats to run down a runway for food and then gave them an injection of either morphine or LiCl. The rats treated with morphine increased their running speed but consumed less food in the goal box, suggesting a positive reinforcement of place learning and aversive taste learning. One possible explanation of this behaviour pattern is that the rats acquired an LTA with the food as the CS and the aversive effect of the morphine as the US, and that the instrumental behaviour of running down the runway was rewarded by the positive affective actions of morphine.

The rats treated with LiCl decreased their running speeds and their food consumption, suggesting aversive associations involving both place and taste. Given the evidence for the independence of the two associations with the effects of morphine, it is possible that there were also two independent associations with aversive effects of LiCl. In an unpublished LTA experiment similar to the present one in which the Equal CS had been paired with a low dose of morphine as the US, the rats had high call ratios when placed into the test box but low ratios whenever they approached the vicinity of the drinking spout. This is consistent with the idea that the rats in the present experiment could have acquired independent aversive associations with the location of the spout (place) and the taste of the CS.

Further evidence consistent with the separation of taste and context conditioning was provided by Desmedt, Hazvi and Dudai (2003), who found that c-AMP-response element-binding protein (CREB), which is crucial for long-term memory, was activated

in insular cortex and lateral septum, but not in parietal cortex and medial septum in rats trained on a taste aversion. In contrast rats trained on a conditioned context aversion had increased CREB activation in the parietal cortex and medial septum but not the insular cortex or the lateral septum. There is also considerable evidence that the hippocampus is a necessary structure for the context learning, as demonstrated in the fear conditioning literature (Frankland et al., 2004; Rudy, Barrientos, & O'Reilly, 2002; Rudy & O'Reilly, 1999, 2001). However it should be noted that this is true only when the context is a small box as is typically used in fear and taste conditioning (and in the present experiment); it is not the case when the context is a large spatial environment (Gaskin & White, 2007).

In the present experiment the observed taste avoidance could be an expression of a combination of environmental context learning (place avoidance) and conditioning (taste avoidance). The BLA lesions would have eliminated the taste avoidance and the environmental conditioning would account for the attenuated avoidance that survived the lesions.

Context-Dependent Retrieval

The attenuated LTA in the D/MD condition could also be an expression of context-dependent retrieval. This theory postulates that internal motivational states such as hunger and thirst are contextual cues that result in the recall of stored situational information relevant to the contextual cue (Hirsh, 1974; Heyman & Bouzas, 1980). In this case the strong motivational state during training is a condition that recruits the amygdala and particularly the BLA. Since the BLA is essential for learning this type of association the behaviour is impaired in its absence if the motivational state remains the same (the D/D condition). However, if the motivational state is changed, in this case from strong to mild deprivation, an alternate system, other than the amygdala, that

learned the information independently of the motivational state is able to express the behaviour (Stouffer & White, 2007). This suggests that the amygdala and the alternate system learn in parallel; since the two forms of learning promote the same behavior (decreased drinking) the two systems work in cooperation. The two systems operate simultaneously; and only under specific circumstances can one prevail and express the behaviour. In the case of the D/MD condition, in the absence of the amygdala. it is possible that the avoidance of the area of the drinking tube expressed by the alternate system does not eliminate drinking as completely as a conditioned aversion to the taste itself, resulting in an attenuated LTA.

This phenomenon is also known as state-dependent learning, in which the retrieval of newly acquired information occurs only if the subject is in the same sensory context and physiological state as during the encoding phase (izquierdo & Dias, 1983; Khavandgar, Homayoun, Torkaman-Boutorabi, & Zarrindast, 2002; Rosenkranz & Johnston, 2007; Slot Bruins & Colpaert, 1999; Zarrindast, Jafari, Ahmadi, & Djahanguiri, 2004). In the D/MD condition the internal state at the time of the encoding of the memory is different from that at the time of testing. It is possible that the restoration of the deprivation state in the same rats could allow them to express the avoidance. This could be tested in future experiments.

MD/MD and MD/D Conditions

Both the absence of deprivation during training and the lack of effect of amygdala lesions suggest that the LTA observed in these conditions was not due to either Pavlovian conditioning or instrumental learning. In support of the idea that different kinds of learning occurred in the strong and mild deprivation conditions, the BLA lesions eliminated the low call ratios in the former, but had no effect on them in the latter

condition. This raises the question of what kind of learning produced the observed taste avoidance when the rats were mildly deprived during training.

Assuming that the small amount of drinking during the training trial by rats in the mild motivational state was not due to deprivation, and that the solution did not, therefore, act as a US (or reinforcer), the learning that occurred in this case could be a form of irrelevant incentive latent learning about the relationship between the taste of the solution and the LiCl-produced illness (see Introduction for a discussion of this type of learning). In this form of learning the rats would have learned to "expect" that an aversive event would follow the taste when they licked the tube on the test trial. However, since no conditioning had taken place, they would not have experienced gastric aversion itself, but a state of anticipatory fear of gastric aversion. This situation has also been described by saying that, for the rat, the taste 'predicts' the aversion (see Dickinson, 1989 for a review). The rats' failure to drink can then be seen as a case of instrumental learning in which the rats acquire behaviors (avoiding the spout) that decrease or eliminate the fear of the predicted aversive state. Thus, this form of the LTA can be called the instrumental taste aversion (ITA). In this case, the reduced drinking and call ratios are due to learning about how to avoid the consequences of drinking or entering the context in which the aversive event occurred.

In the discussion of latent learning in the Introduction data were reviewed showing that appetitive latent learning occurs in the absence of a motivational state, but that deprivation is required for the latent information to affect behaviour on the subsequent test trial. In appetitive learning situations, the motivational state can be controlled by depriving a rat of access to some required substance, such as food, water or salt. In aversive conditioning situations such as that in the present study there are two

sources of motivation: deprivation and the aversive event. In the present experiment even when the motivational state was mild during training (MD) the rats experienced the aversive gastric consequences of the LiCl injection. The failure of rats trained in this condition to acquire an amygdala-based CTA suggests that the deprivation state must be present when the rats experience the CS (the taste of the solution) for it to enter into a conditioned association that allows it to elicit a UR resembling the US (gastric aversion in this case). Furthermore, the acquisition of a non-amygdala-based CTA by rats in a mild state of deprivation shows that the CS was nevertheless able to participate in a latent association with the aversive event.

The expression of ITA during the test trial in the MD/D condition is consistent with the need for a strong motivational state for expression of the latently learned association. Although the ITA expressed by the rats in the MD/MD condition may seem inconsistent with this requirement, it can be suggested that the fear-like aversive state evoked by the taste of the solution may have provided the motivational state thought to be required for expression of the latent learning.

Latent learning is impaired by pre-training lesions of entorhinal cortex, dorsal hippocampus, or ventral hippocampus, but not by pre-training lesions of the fimbria-fornix or lateral amygdala (Coutureau et al., 2002; Gaskin, 2007; Holman, 1980; Khavari & Eisman, 1971; Stouffer & White, 2006; Thistlethwaite, 1951a, 1951b). In one of these experiments Stouffer and White (2006) found that pre-training lesions of the lateral amygdala impaired a conditioned, but not a latent cue preference for salt, reflecting the role of the amygdala in Pavlovian conditioning but not latent learning. On the other hand lesions of the entorhinal cortex and the ventral or dorsal hippocampus impaired the latent, but not the conditioned cue preference for salt.

This hippocampus/entorhinal cortex system is a major conduit of sensory information from the cortex (Amaral & Witter, 1989). The entorhinal cortex/ hippocampus circuitry is involved in a variety of learning and memory tasks, including spatial learning (Aggleton et al., 2000; Fyhn, et al., 2004; Steffenach et al., 2005), nonspatial learning (Jarrard et al., 2004; Hargreaves et al., 2005), inhibitory avoidance learning (Izquierdo & Medina, 1993; Izquierdo et al., 1993; Pereira et al., 2001), and attention (Burwell, 2000). Accordingly, it can be predicted that lesions of the entorhinal cortex would impair ITA learning. This issue will be addressed in future studies.

Several other neural systems could also participate in either the latent learning of the relationship between taste and the aversive effect of LiCl, or in the instrumental learning that results in the ITA. Although they are not in the majority, there are a few reports that amygdala lesion do not impair taste avoidance learning while lesions of other neural structures do have this effect. Insular cortex lesions are known to impair latent learning of taste avoidance (Bernstein & Koh, 2007; Cubero et al., 1999; Dunn & Everitt, 1988; Ferreira et al., 2006; Fresquet, Angst, & Sandner, 2004; Norgren, 1995; Roman & Reilly, 2007). Lesions of the parabrachial nucleus (Bernstein & Koh, 2007; S. Reilly, Grigson, & Norgren, 1993; Yamamoto et al., 1995) also block ITA, while lesions of the central nucleus of the amygdala yield varying results (Bernstein & Koh, 2007; Everitt et al., 2003; Morris et al., 1999; Parkinson et al., 2000).

Conclusion

The present data are consistent with the view of multiple memory systems that can function independently or in competition with each other during the learning of a complex task. In certain specific conditions the BLA plays a critical role in learned taste avoidance when there is a strong motivational state (severe deprivation coupled with one

fluid option). However, at least two other ways in which the LTA could be learned, instrumentally and latently, have been discussed. The present study also investigated the ultrasonic vocalizations produced by rats in during the various phases on taste avoidance learning. The USVs paralleled the amounts drunk in normal rats showing a high call ratio that reflects a positive affect when the rats were consuming more of the palatable substance than water. However BLA lesions disrupted USV production in situations where the rats were severely deprived during training and therefore the taste association was learned through Pavlovian conditioning. These same lesions had no effect when the rats were only mildly deprived. This finding suggests a clear link between affect, motivational state and learning.

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