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Orofacial and Somatic Responses Elicited by Lithium-, Nicotine- and Amphetamine-Paired Sucrose Solution

LINDA A. PARKER[1] AND THERESA CARVELL

University of New Brunswick, Saint John

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PARKER, L A AND T CARVELL *Orofacial and somatic responses elicited by lithium-, nicotine- and amphetamine-paired sucrose solution* PHARMACOL BIOCHEM BEHAV 24(4) 883–887, 1986 —Amphetamine and nicotine share the characteristics of both producing a conditioned taste avoidance response (CTA) via their action within the blood-brain-barrier (BBB) whereas lithium produces a CTA via its action outside of the BBB Although, at the doses employed, all three drugs produced equally strong CTAs, amphetamine (3 mg/kg)- and nicotine (1 mg/kg)-paired 0 5 M sucrose solution elicited a similar pattern of orofacial and somatic responses which differed from that pattern elicited by a lithium (127 2 mg/kg)-paired sucrose solution Sucrose paired with either amphetamine or nicotine elicited suppressed ingestion responses of tongue protrusion and paw licking, but did not elicit enhanced rejection responses On the other hand, sucrose paired with lithium elicited not only suppressed ingestion responses, but also a pattern of enhanced rejection responses of chin rubbing and paw treading The results suggest that the CTA established by lithium is qualitatively different than the CTA established by amphetamine or nicotine

Conditioned taste avoidance Conditioned taste aversions Taste reactivity test Lithium Amphetamine Nicotine Palatability Classical conditioning Psychopharmacology Conditioned drug effects

DRUGS from a wide variety of pharmacological classes can produce a conditioned avoidance response when paired with a flavored solution, (e.g., [7]). However, the standard conditioned taste avoidance test (CTA) measure does not differentiate among pharmacologically dissimilar drugs because most agents effectively produce suppression of drinking of a flavored solution with which they were previously paired. Since both lithium and amphetamine elicit a conditioned avoidance response, Parker [13,14] investigated the nature of the somatic CRs elicited by either lithium-paired flavors or amphetamine-paired flavors to determine whether or not they were similar. Parker [13,14] found that rats injected with lithium demonstrated a chin rub CR, whereas those injected with amphetamine did not show a chin rub CR. This finding suggests that these drugs possess different sensory qualities which become associated with the flavored solution

A chin rub response is also elicited by unconditioned bitter tasting quinine solutions [10]. In the taste reactivity test, described by Grill and Norgren [10] and Berridge, Grill and Norgren [3], palatable taste stimuli, such as a sucrose solution, elicited a distinctive pattern of ingestive responses On the other hand, unpalatable taste stimuli, such as quinine solution, elicited a distinctive pattern of rejection responses which facilitated the removal of the taste stimuli from the oral cavity Among the rejection pattern of responses are chin rubs Because of the similarity in response patterns that rats demonstrate to a bitter quinine solution and to a lithium-paired flavored solution, Garcia, Hankins and Rusiniak [8] and Grill and Norgren [10] suggested that the flavor avoidance produced by lithium is mediated by a hedonic shift in the palatability of the flavored solution. That is, the flavored solution becomes distasteful to the rat due to its association with lithium. The absence of chin rubs when amphetamine serves as the US drug suggests that a palatability shift does not mediate an amphetamine-based CTA

Smith and Parker [18] measured somatic CRs elicited by flavored solutions paired with each of a variety of drug agents, the agents employed were apomorphine, scopolamine, physostigmine, methylscopolamine and neostigmine Each of the agents employed was capable of producing chin rub CRs Since methylscopolamine and neostigmine are incapable of crossing the blood-brain-barrier (BBB), the authors suggested that chin rub CRs may be produced by activation of a mechanism which is sensitive to agents which act outside of the BBB This possibility is especially interesting because, although lithium chloride is capable of crossing the blood-brain-barrier, Smith [17] has demonstrated that a

[1]Requests for reprints should be addressed to Linda A Parker, Division of Social Science, P O Box 5050, University of New Brunswick, Saint John, N B E2L 4L5

lithium based CTA is mediated by the peripheral, rather than the central, effects of lithium. Smith [17] reported that intraperitoneal (IP) administration, but not intracerebroventricular (ICV) administration, of lithium produced a CTA. The plasma lithium concentration was higher 0.5, 1.5 and 4 hr after the injection when lithium had been administered IP than when it had been administered ICV, while the reverse was true for the brain and cerebrospinal fluid (CSF) lithium concentration. Lithium-induced CTAs appear to be mediated by activation of the area postrema which is sensitive to blood-borne toxins and may be activated by agents which are incapable of crossing the BBB [6]. On the other hand, although amphetamine has both peripheral and central effects, depletion of the central dopaminergic system, by ICV injections of 6-Hydroxydopamine [20] or by dorsolateral tegmental lesions [21], prevents the establishment of amphetamine-induced CTAs, but not lithium-induced CTAs. Furthermore, para-hydroxyamphetamine which penetrates the BBB poorly is much weaker than amphetamine in producing a CTA [5]. These findings have led investigators to suggest that amphetamine produces its CTA predominantly by means of the activation of a mechanism within the BBB (see [7]).

Nicotine is another peripherally and centrally acting drug which appears to produce a CTA by means of the activation cholinergic systems within the blood-brain-barrier [11]. A nicotine-induced CTA was blocked by mecamylamine, a ganglion-blocking drug which penetrates the CNS, but was not blocked by hexamethonium, a ganglion-blocking drug which penetrates the BBB poorly. Since the peripheral blocking agent did not block the development of a nicotine-induced CTA, Kumar *et al.* [11] suggested that the central, rather than the peripheral, actions of nicotine were responsible for producing a CTA. Therefore, since nicotine and amphetamine may produce a CTA by means of activation of a mechanism within the BBB, we were interested in determining whether they would also produce similar orofacial and somatic CRs when the drug paired flavors were presented in the Taste Reactivity Test (TRT) presented by Grill and Norgren [10] and later refined by Berridge and Grill [4].

The taste reactivity test has shown that the pattern of orofacial and somatic responses elicited by flavored solutions differs on the basis of their palatability (e.g., [4]). A highly palatable sweet sucrose solution elicits an ingestion pattern of responses which includes tongue protrusions and paw licking. An unpalatable bitter quinine solution elicits a rejection pattern of responses which includes chin rubbing, gaping and paw treading. Flavored solutions that have previously been paired with lithium chloride elicit a pattern of rejection responses that resembles those elicited by bitter quinine solutions (e.g., [10]). In the following experiment, the above ingestion and rejection responses were used to classify the pattern of orofacial and somatic CRs elicited by a nicotine-, amphetamine- or lithium-paired sucrose solution.

METHOD

Subjects

Fifty-four male Sprague-Dawley rats, obtained from Charles River Labs, Quebec, were housed individually in stainless steel cages in a room with a 12 hr ON/OFF light schedule. The rats weighed 278–325 g on the first conditioning day. For the duration of the experiment, the subjects were maintained on ad lib access to Purina Rat Chow and water except where stated otherwise.

Apparatus

Adaptation and testing trials took place in a testing room which contained the glass test chamber (22.5×26×20 cm), the chamber was covered with a Plexiglas ceiling. To facilitate viewing of the rat's ventral surface, a mirror was located at an angle below the chamber. A videocamera was also focused on the mirror to monitor the rat's orofacial and somatic responses. A Hitachi HV-62 videocamera transmitted the image through a videocassette recorder (JVC-CR 6060 U) in an adjacent room to an Electrohome 17 in monitor.

Procedure

Surgery. One week after their arrival in the laboratory, the rats were surgically implanted with intraoral cannulae as described by Parker [12]. The rats were permitted to recover for a period of at least 3 days, during which they had free access to food and water. On the final recovery day, the cannula of each rat was flushed with water to prevent blockage by food particles.

Conditioning trials. The 54 rats were randomly assigned to six groups: CS+ Lithium (n=10), CSc Lithium (n=9), CS+ Nicotine (n=9), CSc Nicotine (n=8), CS+ Amphetamine (n=9), CSc Amphetamine (n=9). The rats received three conditioning trials, with one day intervening between each trial.

During the conditioning trials, each rat had a 1 M long infusion hose connected to its cannula. The rat then received a 5 ml intraoral infusion of 0.5 M sucrose solution over a 5 min period at a rate of 1 ml/min. Immediately after the sucrose infusion, each rat received an intraperitoneal (IP) injection of an appropriate drug solution. The rats in the CS+ groups received 127.2 mg/kg of 0.15 M lithium chloride in solution with distilled water at a volume of 20 ml/kg, 3.0 mg/kg of d-amphetamine in solution with saline at a volume of 18 ml/kg, or 1.0 mg/kg of nicotine in solution with saline at a volume of 18 ml/kg. The rats in the CSc groups were injected IP with 18 ml/kg of physiological saline immediately after the sucrose infusions.

Twenty four hours after each conditioning trial, the rats in the control groups CScL, CScA and CScN received a 127.2 mg/kg IP injection of lithium, a 3 mg/kg IP injection of amphetamine or a 1 mg/kg IP injection of nicotine, respectively, but these injections were not paired with the sucrose infusion. These injections were matched by an 18 ml/kg IP saline injection in the CS+L, CS+A and CS+N groups of rats.

Adaptation trials. Adaptation to the test chamber began three days after the final conditioning trial and continued for each of three consecutive days prior to the test trial. During each adaptation trial, each rat was removed from its home cage and placed in the test chamber. Once the rat was inside the chamber a 1 m long infusion hose was connected to its cannula. One minute after the hose was in place, each rat received a 2 ml infusion of water over 2 min via a Sage Infusion Pump set at a flow rate of 1 ml/min. The rat was then returned to its home cage and the test chamber was cleaned and dried.

Taste reactivity test trial. On the day following the final adaptation trial, the rats were tested for orofacial and somatic CRs elicited by sucrose solutions. Each rat was placed in the test chamber and, one minute later, it received an intraoral infusion of 0.5 M solution of sucrose for 2 min at a rate of 1 ml/min. The rats' responses were videotaped at this time.

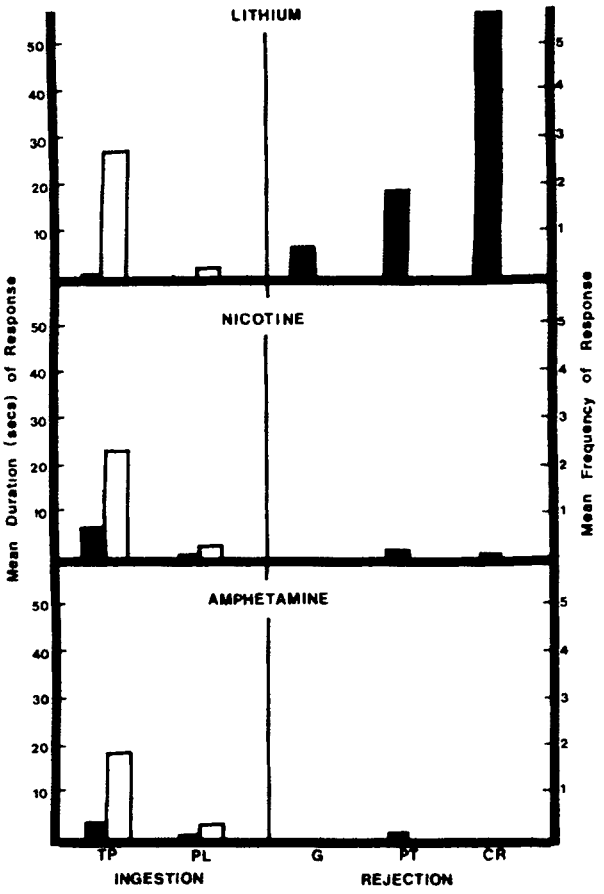

FIG 1 Mean duration (secs) of the ingestion responses of Tongue Protrusions (TP) and Paw Licking (PL) and mean frequency of the rejection responses of Gaping (G), Paw Treading (PT) and Chin Rubbing (CR) elicited by sucrose infusions in the CS+ (closed bars) and the CSc (open bars) Groups which received Lithium, Nicotine and Amphetamine during the conditioning trials

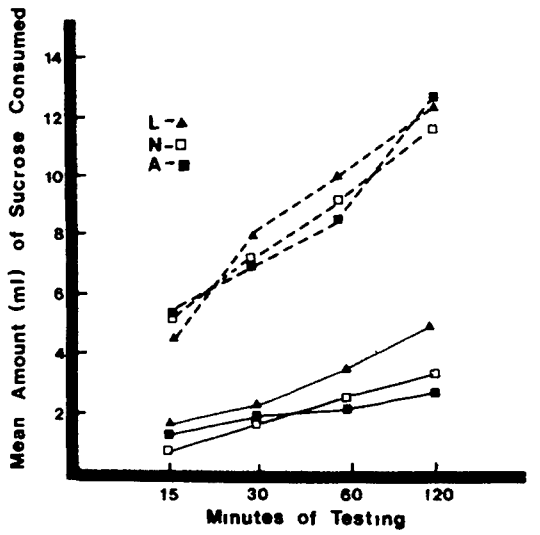

FIG 2 Mean cumulative amount (ml) of sucrose solution consumed by the various groups at each of intervals 15, 30, 60 and 120 min The solid lines depict the CS+ groups and the broken lines depict the CSc groups The US drug employed was Lithium (▲), Nicotine (□ or Amphetamine (■)

The orofacial and somatic responses that were measured were among those described by Berridge and Grill [4] The two patterns of responses which we will report were an ingestion and a rejection pattern. The ingestion pattern of responses which we measured included tongue protrusions and paw licks Tongue protrusions (TP) may be rhythmic and symmetrical with the tongue covering the upper incisors or nonrhythmic lateral protrusions of the tongue on either side of the mouth. Paw licking (PL) is defined as the paws being held close to the mouth and lapped.

The rejection pattern of responses which we measured includes the orofacial response of gaping and the somatic responses of chin rubbing and paw treading. Gaping (G) involves the rapid large-amplitude opening of the mandible with the corners of the mouth retracting, forming a triangular shaped mouth The somatic response of chin rubbing (CR) is defined as the lowering of the head which brings the mouth into direct contact with the floor or the wall and projecting the body forward. Paw treading (PT) is defined as the rat extending one forelimb forward against the floor and retracting the other back, actively rubbing the forepaws on the floor

Flavor avoidance test trial Twenty-four hr after the taste reactivity test trial, each rat was deprived of water for 2 hr before being presented with a weighed water bottle filled with a 0 5 M solution of sucrose. The amount that each rat consumed was measured at 15 min, 30 min, 60 min and 120 min.

Data analysis. The videotaped records of each rat's responses during the taste reactivity test trial were scored by a rater blind to the experimental conditions The data for each orofacial or somatic response was analyzed as a 2×3 ANOVA with the factors of CS condition (CS+, CSc and the US condition (lithium, nicotine and amphetamine). The flavor avoidance test data was analyzed as a 2×3×4 repeated measures ANOVA for intervals 15 min–120 min with the factors of CS condition × US condition × Test intervals. For all statistical tests, the criterion. level was $p<0$ 05.

RESULTS

Taste reactivity test. Figure 1 presents the mean duration (secs) or the mean frequency of each behavior scored when the US drug was lithium, nicotine or amphetamine The closed bars depict the CS+ groups and the open bars depict the CSc groups. The data in the lefthand section of Fig. 1 represent the mean duration (secs) of ingestion responses and the data in the righthand section of Fig. 1 represent the mean frequency of rejection responses.

The two ingestion responses of Tongue Protrusions (TP)

and Paw Licking (PL) showed evidence of modification through conditioning The 2×3 ANOVA of both the TP and the PL data revealed a significant CS Condition effect, TP $F(1,48)=40\ 7$, $p<0\ 001$, PL $F(1,48)=6\ 9$, $p<0\ 01$ The CS+ Groups spent less time demonstrating tongue protrusions and paw licking than did the CSc groups regardless of the US drug condition No other main effects or interactions were significant.

The three rejection responses measured were the mean frequency of Gaping (G), Paw Treading (PT) and Chin Rubbing (CR) Only the PT and CR measures showed evidence of conditioning The 2×3 ANOVAs revealed a significant CS Condition × US Condition interaction for the paw treading scores, $F(2,48)=4\ 05$, $p<0\ 025$, and for the chin rubbing scores, $F(2,48)=120\ 5$, $p<0\ 001$ For both measures, subsequent t-tests (using a pooled error term) revealed that Group CS+L showed a greater frequency of responding than all other groups (p's$<0\ 01$)

Flavor avoidance test The mean cumulative amount of 0 5 M sucrose solution consumed at each interval of testing is presented in Fig 2 The solid lines represent the CS+ groups and the broken lines represent the CSc groups A 2×3×4 repeated measures ANOVA revealed a significant CS Condition effect, $F(1,48)=47\ 6$, $p<0.001$, and a significant CS Condition × Intervals effect, $F(3,144)=25\ 4$, $p<0\ 001$ The CS+ groups drank less sucrose overall than did the CSc groups and the difference increased across intervals of drinking However, the groups did not differ on the basis of the US drug condition The strength of the avoidance response did not differ among the different CS+ groups

DISCUSSION

Although lithium, nicotine and amphetamine produced conditioned flavor avoidance responses which were equivalent in strength, they produced different taste reactivity responses When amphetamine or nicotine were the US drugs, the ingestion response of tongue protrusions was suppressed, but no rejection responses were observed On the other hand, when lithium was the US drug not only were the ingestion responses of tongue protrusions suppressed, but also the rejection responses of chin rubbing and paw treading were enhanced

The results clearly indicate that amphetamine- and nicotine-paired sucrose solution elicits a similar pattern of orofacial and somatic responses which differs from the pattern of responses elicited by lithium-paired sucrose solution Although each drug-paired flavor elicited suppressed ingestion responses, only the lithium-paired flavor elicited the rejection pattern of responses Since the suppression of ingestion responses probably reflects a similar process which is measured by the avoidance test, the suppression of these responses did not differentiate among the drugs that were employed The enhancement of rejection responses, however, did differentiate among the drugs If the occurrence of the rejection pattern indicates a palatability shift, as has been suggested by Grill and his colleagues [2, 3, 4, 10], one can argue that the avoidance of nicotine- and amphetamine-paired flavors is not mediated by a palatability shift

One of the characteristics that amphetamine and nicotine share is that they are drugs which animals will self-administer (e g , [9]) Smith and Parker [17] suggested that CTAs produced by such positively reinforcing drugs may not be mediated by a palatability shift of the drug-paired flavored solution and, therefore, not support chin rubs The results of the above experiment support this suggestion, since the amphetamine- and nicotine-paired flavored solution did not elicit the rejection pattern of orofacial and somatic CRs that are elicited by unpalatable flavored solutions

Another shared characteristic of amphetamine and nicotine is that they both may produce CTAs by means of their action within the blood-brain-barrier (BBB) rather than their action outside of the BBB [5, 7, 11, 20, 21] Smith and Parker [18] reported that peripherally acting methylscopolamine and neostigmine effectively produced a CTA and chin rub CRs Methylscopolamine, as well as lithium, appears to produce a CTA by activation of the area postrema [2,19] Recent reports suggest that lesions of the area postrema modify the palatability of sucrose flavored solutions (e g , [15]) Since the area postrema is the chemoreceptive trigger zone sensitive to peripheral toxins and has been demonstrated to play a role in the processing of palatability information, this may be the site responsible for the palatability shift (as indicated by chin rub CRs) produced by pairing some drugs with a flavored solution Neither amphetamine nor nicotine, which are purported to produce a CTA by means of their central rather than their peripheral action [5, 7, 11, 20, 21], supported the conditioning of chin rub CRs

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