Neuroscience 123 (2004) 635–645

NEURONAL ACTIVITY IN THE NUCLEUS ACCUMBENS IS NECESSARY FOR PERFORMANCE-RELATED INCREASES IN CORTICAL ACETYLCHOLINE RELEASE

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Abstract—*In vivo* microdialysis was used to determine the necessity of neuronal activity in the nucleus accumbens (NAC) for task-induced increases in cortical acetylcholine (ACh) efflux. Rats were trained in a behavioral task in which they were required to perform a defined number of licks of a citric acid solution in order to gain access to a palatable, cheese-flavored food. Upon reaching a consistent level of performance, rats were implanted with microdialysis cannula in the medial prefrontal cortex (mPFC) and either the ipsilateral shell of the NAC or in the dorsal striatum (STR; control site). Dialysis samples from the mPFC were analyzed for ACh concentrations and samples from the NAC were analyzed for dopamine (DA) concentrations. Performance in the task was associated with increases in both ACh efflux in the cortex (150–200%) and DA efflux in the NAC (50–75%). These increases were blocked by administration of tetrodotoxin (TTX; 1.0 μM) via reverse dialysis into the NAC. Administration of TTX into the dorsal STR control site was ineffective in blocking performance-associated increases in cortical ACh. The D2 antagonist sulpiride (10 or 100 μM) administered into the NAC via reverse dialysis was ineffective in blocking increases in cortical ACh efflux. The present data reveal that neuronal activity in the NAC is necessary for behaviorally induced increases in cortical ACh efflux and that this activation does not require increases in D2 receptor activity.

Key words: dopamine, TTX, reward, microdialysis, prefrontal cortex, incentive.

The basal forebrain cholinergic system (BFCS) projects to practically all areas and layers of the cerebral cortex (Woolf and Butcher, 1986; Woolf, 1991) and is critically involved in the mediation of attentional functions such as the detection, selection, and processing of stimuli (for reviews see Everitt and Robbins, 1997; Sarter and Bruno, 1997; Sarter et al., 2001). Several decades of psychopharmacological research in laboratory animals (Everitt and Robbins, 1997; Sarter and Bruno, 1997; Sarter et al., 2001) and in humans (Dunne and Hartley, 1986; Vitiello et al., 1997; Witte et al., 1997; Mancuso et al., 1999; Thiel et al., 2002) have substantiated this role for cholinergic transmission in attentional processes. Numerous studies have demonstrated that activity within the BFCS is necessary for performance in tasks designed to explicitly measure attention (Muir et al., 1992, 1994; McGaughy et al., 1996). In addition, recent experiments have revealed that performance in attentional tasks is sufficient to increase cortical ACh efflux (Himmelheber et al., 2000, 2001; Passetti et al., 2000; Dalley et al., 2001; Arnold et al., 2002). Based on these data, some have suggested that impairments in attentional processing could contribute to the development of cognitive deficits in a variety of neuropsychiatric disorders (Jones et al., 1991; Greenwood et al., 1993; Berntson et al., 1998; Sarter and Bruno, 1999).

These hypotheses have promoted interest in neuronal systems that regulate the excitability of the basal forebrain corticopetal cholinergic projections, particularly by the medium spiny GABAergic projection originating from the shell region of the nucleus accumbens (NAC; Zaborszky and Cullinan, 1992; Zahm and Heimer, 1993). The functional modulation of basal forebrain activity by this pathway was first demonstrated in the classical work of Mogenson and colleagues (Yang and Mogenson, 1984, 1989) in anesthetized rats. Although the precise circuitry underlying the ability of NAC efferents to regulate the excitability of the BFCS remains unresolved (see Zahm et al., 1999) recent studies have demonstrated that pharmacological manipulations of neurotransmission within the NAC modulate cortical acetylcholine (ACh) efflux (Moore et al., 1999; Neigh-McCandless et al., 2002).

If, as proposed, the NAC serves to integrate cortical and limbic information in order to modulate subsequent stimulus selection and processing, then one might expect that performance in certain behavioral tasks would reveal a concomitant activation of accumbens and basal forebrain. A test of the functional relationships between these two regions during performance requires a task that markedly enhances accumbens and basal forebrain activity. The literature suggests that behavioral procedures that would be most effective in producing marked and persistent activation of these two regions would be characterized by specific task attributes, including: highly activating and arousing conditions (Muir et al., 1995; Sarter and Bruno, 2000), performance on operant schedules (Cousins et al., 1999), the requirement that the subject ac-

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Abbreviations: ACh, acetylcholine; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BFCS, basal forebrain cholinergic system; DA, dopamine; Glu, glutamate; mPFC, medial prefrontal cortex; NAC, nucleus accumbens; STR, dorsal striatum; SULP, sulpiride; TTX, tetrodotoxin.

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doi:10.1016/j.neuroscience.2003.10.006

tively 'track' stimuli associated with the conditionality and response components of the task (Sarter et al., 2001), an anticipatory component generated by explicit delays between task onset and presentation of reward to enhance the motivational levels (Inglis et al., 1994), and a significant performance component necessary for reinforcement, perhaps including the performance of a non-preferred response in order to gain access to the reinforcer (in order to further tax the processing of motivational information, see Berridge and Robinson, 1998). The fact that none of these attributes alone is sufficient for coupled activation of the NAC and frontal cortex has been demonstrated in several recent publications (Inglis et al., 1994; Acquas et al., 1996; Himmelheber et al., 2000; Neigh et al., 2001; Arnold et al., 2002).

The task used in the present study was designed to operationalize certain features of Robinson and Berridge's (1993) theory about the role of NAC dopaminergic transmission in the attribution of incentive salience to stimuli. Importantly, the purpose of the behavioral procedure used in this experiment was not to further demonstrate a role of NAC dopamine (DA) in these processes or even to initiate an analysis of the contributions of the BFCS to the mechanisms described by this theory, but to generate a behavioral procedure that concurrently and robustly activates NAC dopaminergic and cortical cholinergic transmission. Having identified such a procedure, the goal was to then determine the necessity of neuronal transmission in the NAC for task-induced increases in cortical ACh efflux. In essence, animals were trained to consume a mildly aversive citric acid solution (0.05%) in order to gain access to a highly palatable food reward. The first aim of the study was to establish the effectiveness of this procedure to elicit concomitant increases in both dopaminergic transmission in the NAC and cholinergic transmission in the mPFC as measured by dual probe microdialysis. Once these changes were established, tetrodotoxin (TTX), a fully reversible but potent and specific blocker of voltage-gated sodium channels, was used to temporarily inactivate Na^+-gated transmission within NAC in order to assess the necessity of such transmission in the NAC for increases in cortical ACh efflux. Furthermore, the anatomical specificity of the TTX manipulation was tested by perfusing TTX into the overlying dorsal striatum (STR) of animals engaged in the same behavioral task and again measuring cortical ACh efflux. Finally, we determined whether reductions in D2 receptor activity, that would be expected to occur following local perfusion of TTX, contributed to the TTX effect by measuring the effects of intra-NAC perfusions of the D2 receptor antagonist sulpiride (SULP) on task-induced increases in cortical ACh efflux.

EXPERIMENTAL PROCEDURES

Animals

Adult male Fisher-344/Brown Norway F1 hybrid rats (Harlan Sprague–Dawley, Indianapolis, IN, USA), weighing between 300 and 400 g, served as subjects in this experiment. Animals were maintained in a temperature-controlled environment on a 12-h light/dark cycle (lights on at 06:30 h). Beginning at the onset of training, animals were kept on a restricted food and water schedule of 14.0 g of rat chow (Laboratory Diet/Rodent; Harlan Teklad, Madison, WI, USA) and 1.5 h of water daily, with their body mass maintained between 85% and 95% of free feeding body-weights. Prior to surgery animals were housed in individual stainless steel hanging cages. One day before microdialysis guide cannula implantation, animals were moved to individual plastic cages with corncob bedding (Harlan Teklad) where they were housed for the duration of the study. The minimum number of animals possible to provide sufficient statistical power were used in this study. Animal care, relief from suffering, and experimentation were performed in accordance with protocols approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee and consistent with the NIH Guide for the Care and Use of Laboratory Animals.

Behavioral training procedures

Rats were placed in a concentric dialysis bowl (35 cm height×38 cm depth×38 cm diameter at the upper rim; CMA, Stockholm, Sweden) for 1–4 h prior to transfer to the test chamber. The test chamber was constructed of clear acrylic walls with a stainless steel rung floor (42 cm×40 cm×47 cm; l×w×h) and was divided in half, creating two compartments, with a wire mesh screen that could be removed by pulling it out from the outside of the chamber. One compartment (henceforth termed "compartment A") was equipped with a lickometer (Med Associates, Inc., St. Albans, VT, USA). Twenty-five minutes after transfer of the animal to this compartment, the lick spout was extended. The 25 min delay period was based on the finding that any transfer-related increases in cortical ACh efflux return to baseline within that period (Neigh et al., 2001). Rats were shaped to lick the spout delivering a 0.05% citric acid solution (ICN Biomedicals Inc., Aurora, OH, USA). Ultimately, animals were trained to perform 500 licks in order for the mesh screen to be removed and to gain access to palatable, cheese-flavored food (0.5 g; Cheetos; Frito-Lay Inc., Plano, TX, USA) in the opposite compartment (henceforth termed "compartment B"). Animals remained in compartment B for an additional 25 min after crossing over. Three trials were performed per day, and animals were returned to the bowls between trials for 2 min. Once an animal successfully completed the task (500 licks/trial) on 3 consecutive days, guide cannula were implanted.

Surgery

Animals were anesthetized with ketamine (100.0 mg/kg i.p.) and xylazine (3.0 mg/kg i.p.) and placed into a stereotaxic frame. Two thermoplastic resin microdialysis guide cannula (0.72 mm o.d.; Bioanalytical Systems (BAS), West Lafayette, IN, USA) were stereotaxically implanted into the medial prefrontal cortex (mPFC) (AP: 3.0 mm; L: 0.8 mm; V: 1.0 mm from dura at 10° rostral) and shell region of the NAC (AP: 0.3 mm; L: 1.1 mm; V:5.7 mm from dura at 15° caudal; Paxinos and Watson, 1987). In control animals, guide cannula were implanted into the mPFC and the STR (AP: 0.3 mm; L: 2.1 mm; V: 3.7 mm from dura). The position of the guide cannula was fixed with dental cement and skull screws. The two guides were implanted into the same hemisphere, and the selection of the hemisphere for implantation was counterbalanced across animals. A dummy cannula that was flush with the end of the guide was inserted into the guides when animals were not undergoing microdialysis testing. The mPFC was selected based on its well-established role in the mediation of executive functions, including attention (see Sarter and Bruno, 1997, 1999, 2000; Sarter et al., 2001 for reviews) and on previous demonstrations that ACh efflux in mPFC is stimulated during performance of tasks explicitly taxing attentional processes (Arnold et al., 2002; Dalley et al., 2001; Himmelheber et al., 2000, 2001). The shell region of the NAC was selected because it is the source of GABA-containing projections to regions of the basal forebrain containing cholinergic corticopetal projections (Zaborszky and Cullinan, 1992; Zahm and Heimer, 1993) and pharmacological manipulations within the shell region modulate drug-induced changes in mPFC ACh efflux (Moore et al., 1999; Neigh-McCandless et al., 2002). At the

conclusion of the surgery, animals received a prophylactic dose of the antibiotic amoxycillin (30,000 units, subcutaneously). Following surgery, animals were allowed to recover for 3 days during which time they had free access to food and water. On the fourth post-surgical day food and water were again restricted and re-training began on the fifth post-surgical day.

Microdialysis sessions

After recovering from surgery and between microdialysis sessions, animals were retrained until they again reached the 500 lick-criterion for 3 successive days prior to the subsequent microdialysis session. Animals participated in four microdialysis sessions and received a different pharmacological treatment on each of the four sessions that were designed to assess the effects of perfusion of vehicle, TTX, and SULP (two concentrations) into the NAC on performance-associated mPFC ACh and NAC DA efflux. Repeated cortical microdialysis was previously demonstrated to affect neither basal cortical ACh efflux nor the effects of various drug treatments (e.g. Moore et al., 1995a,b).

On each microdialysis test day, animals were allowed to acclimate to the microdialysis bowls for at least 30-min prior to the insertion of concentric microdialysis probes (0.32 mm o.d., 2.0 mm exposed membrane length; BAS) through the guide cannula. Each probe was perfused with artificial cerebrospinal fluid (aCSF; pH 7.2) containing the following (in mM): NaCl, 166.5; $NaHCO_3$, 27.5; KCl, 2.4; Na_2SO_4, 0.5; KH_2PO_4, 0.5; $CaCl_2$, 1.2; $MgCl_2$, 0.8; glucose 1.0. Furthermore, the aCSF perfused through the mPFC probe contained a low concentration of the acetylcholinesterase inhibitor neostigmine bromide (0.05 μM; Sigma Chemical, St. Louis, MO, USA) to enable the reliable detection of basal cortical ACh efflux in dialysates collected over relatively short (5 min) periods. The inlets and outlets of the probes were constructed of FEP tubing (internal volume:1.2 μl/10 cm; CMA) and were attached to a dual channel liquid swivel (Instech, Plymouth Meeting, PA, USA) and perfused for 3 h before collection of dialysates began. Studies have confirmed that following this perfusion interval, basal ACh efflux is stable and highly (>95%) dependent on voltage-gated Na^+ channels (Moore et al., 1992). Probes were perfused at a flow rate of 2.5 μl/min for the duration of the sessions and, following the initial discard period, collections were taken from the mPFC probe every 5 min. Collections from the NAC probe were taken every 10 min to facilitate reliable detection of basal levels of DA. DA collection vials contained 5.0 μl of sodium bisulfite/EDTA as an antioxidant. After 20-min of consecutive baseline collections, perfusion of drugs into the NAC or STR commenced. Animals remained in the baseline environment (bowls) for an additional 10-min before being transferred to compartment A. Twenty-five minutes after transfer, the lick spout was introduced and 5-min later, the dividing screen was removed. The number of licks made and the time it took the animals to reach and consume the food located in the compartment B were recorded. Animals remained in compartment B for an additional 25 min after crossing over. During the microdialysis sessions, time in each compartment was held constant in order to standardize collection intervals across animals and conditions.

Drug treatments

This experiment assessed the effects of intra-NAC perfusion of aCSF, the Na^+ channel blocker TTX (1 μM; Sigma), or the D2 antagonist L-SULP (SULP; 10 or 100 μM; RBI, Natick, MA, USA) on NAC DA and mPFC ACh release and behavior. The concentration of TTX was selected on the basis of pilot studies (see also Lipska et al., 2002; Saulskaya and Mikhailova, 2002). Intra-accumbens administration of 100 μM SULP was previously shown to block the increase in cortical ACh efflux produced by systemic administration of the benzodiazepine partial inverse agonist FG

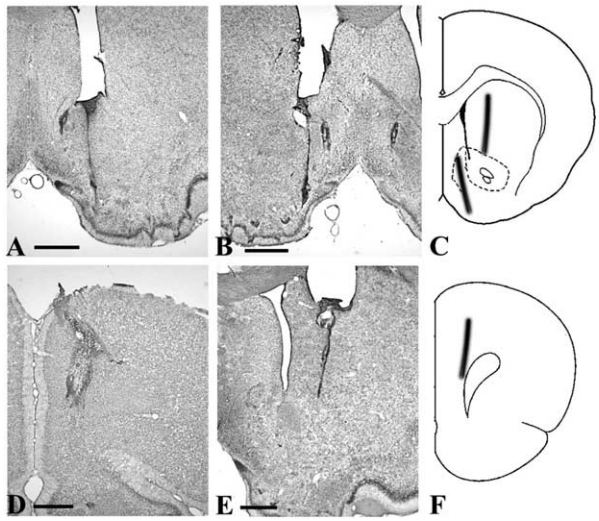

Fig. 1. Photomicrographs depicting representative placements of microdialysis probes in the shell of the NAC (A, B), the mPFC (D) and the STR (E, scale bars=1.0 mm). Representative placements in the accumbens and striatum are schematically shown in C, and placements in the medial prefrontal cortex are represented schematically in F. In A, B and E, the damage produced by the guide cannula (O.D.=0.38 mm) is clearly visible dorsal to the placement of the probes (length=2.0 mm, O.D.=0.24 mm). Probes placed in the medial prefrontal cortex penetrated lower layers of the ventral cingulated cortex and the prelimbic region (D, F).

7142 (Moore et al., 1999), suggesting that this dose is sufficient to attenuate NAC D2 receptor mediation of cortical ACh efflux. In addition, and in a separate group of animals, the possibility that the ability of TTX to block increases in cortical ACh efflux was due to disruption of extra-NAC, striatal neuronal activity, was assessed by infusing TTX via a probe placed in the anterior STR (see Fig. 1). Thus, this experiment was limited to a test of the effects of perfusion of TTX or aCSF into the STR on cortical ACh efflux in animals performing the behavioral procedure.

Verification of probe placement

Following the last microdialysis session, animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.2% heparin in 0.9% saline, followed by 10% formalin. The brains were stored in 10% formalin at 4 °C for 24 h. Brains were then transferred to 30.0% sucrose phosphate buffer for cryoprotection. To verify probe placement, several sections (45 μM) encompassing the dialysis probe placement were stained using Cresyl Violet. Microsopical inspection was done using an Olympus AX microscope (Olympus America, Melville, NY, USA). Microphotographs were obtained using an Olympus Magnafire Digital CCD camera (Model S99806) equipped with a 2/3rds inch chip, attached to the microscope by an Olympus-USPT coupler (Olympus America). Optical magnifications are indicated in the figure legends. Only data from subjects that had both probe placements within the target regions (mPFC and shell region of the NAC for the first experiment, and mPFC and STR for the second experiment) were included in the final analyses.

Neurochemical analyses

ACh. ACh levels in dialysates collected from the mPFC were determined by high-performance liquid chromatography with electrochemical detection. Briefly, 10.0 μl from each collection were injected, and ACh and choline were separated by a C-18 carbon polymer column (250×3 mm; ESA, Inc., Chelmsford, MA, USA)

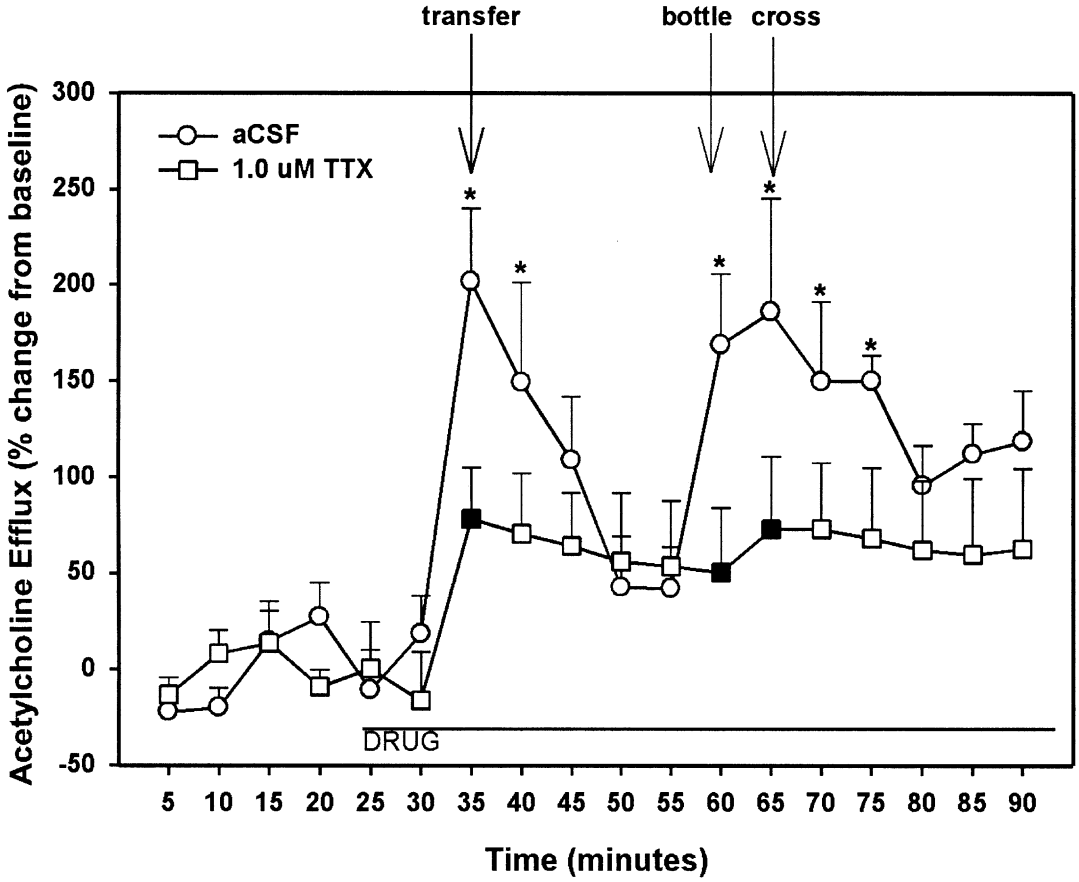

Fig. 2. Effects of NAC perfusion of aCSF ($n=6$) or TTX (1 μM, $n=6$) on mPFC ACh efflux (data in this and other figures depict the means and S.E.M.). The horizontal bar above the abscissa indicates the period aCSF or drug perfusion that commenced immediately after the 4th collection. Transfer of animals from the bowls into compartment A, presentation of the bottle, licking of the citric acid solution, removal of the screen and cross to compartment B and consumption of palatable food significantly increased mPFC ACh efflux over baseline (* $P<0.05$; the arrows in this and the other figures indicate the first collection after the events which occurred 2.5 min prior to the marked collections). Again, these manipulations were designed to produce robust increases in ACh efflux, and not to determine the specific behavioral variables responsible for such increases. NAC perfusion of TTX (1 μM) attenuated the increases in ACh efflux (filled squares [■] denote a significant difference from the aCSF session; $P<0.05$).

using a sodium phosphate mobile phase (in mM: NaH_2PO_4, 100.0; TMACl, 0.5; 1-octanesulfonic acid, 2.0; microbiocide, 0.005%; pH 8.00). ACh was hydrolyzed on a post-column immobilized enzyme reactor and converted to hydrogen peroxide (Potter et al., 1983) that was detected with a 'peroxidase-wired' glassy carbon electrode (Huang et al., 1995). The detection limit for ACh under these conditions was approximately 10.0 fmol/10.0 μl injection.

DA. DA levels in dialysate collected from the NAC were also determined using high-performance liquid chromatography with electrochemical detection. From each sample, 25.0 μl was injected and DA was detected via a dual electrode coulometric detector (ESA, Inc.) with the potential for electrode 1 = −175.0 mV and for electrode 2 = 175.0 mV. DA was separated on a C-18 polymer column (80×4.6 mm; HR-80, ESA, Inc.) using a sodium phosphate mobile phase (in mM: NaH_2PO_4, 75; octanesulfonic acid, 2.0; EDTA, 25; triethylamine 100 μl; methanol, 18.0%; pH 5.6). The detection limit for DA under these conditions was approximately 0.5 fmol/25.0 μl injection.

Statistical analyses

Basal ACh efflux was determined by averaging the values from the first four 5-min collections taken after the discard phase (collections taken at 5–20 min as indicated in Figs. 2, 4 and 5). Basal DA efflux was calculated as the average of the first two 10-min collections after the discard period (see Fig. 3). The stability of basal transmitter efflux (expressed as absolute values) within and across dialysis sessions was assessed for both DA and ACh in each experiment using a two-way repeated measures analysis of variance (ANOVA) with the factors of session and time (time representing the four [ACh] or two [DA] collections prior to the administration of drug into the NAC or STR). To determine the ACh and DA efflux and the effects of drugs perfused through the NAC or STR probes, two-way repeated measures ANOVAs were conducted for each transmitter (expressed efflux as a percent change from baseline) in each experiment with the factors of drug and time (all collections taken while drug was perfused). In the case of a significant result, all pair-wise multiple comparisons were analyzed using Tukey tests. *t*-Tests were used to compare the animals' performance during the aCSF and TTX sessions. A one-way ANOVA was used to compare the performance of the animals during sessions testing the effects of aCSF and SULP (10 μM or 100 μM). α was set at 0.05 for all statistical analyses. The computer software package SigmaStat 2.0 (SPSS Inc., Chicago, IL, USA) was used to conduct all statistical analyses.

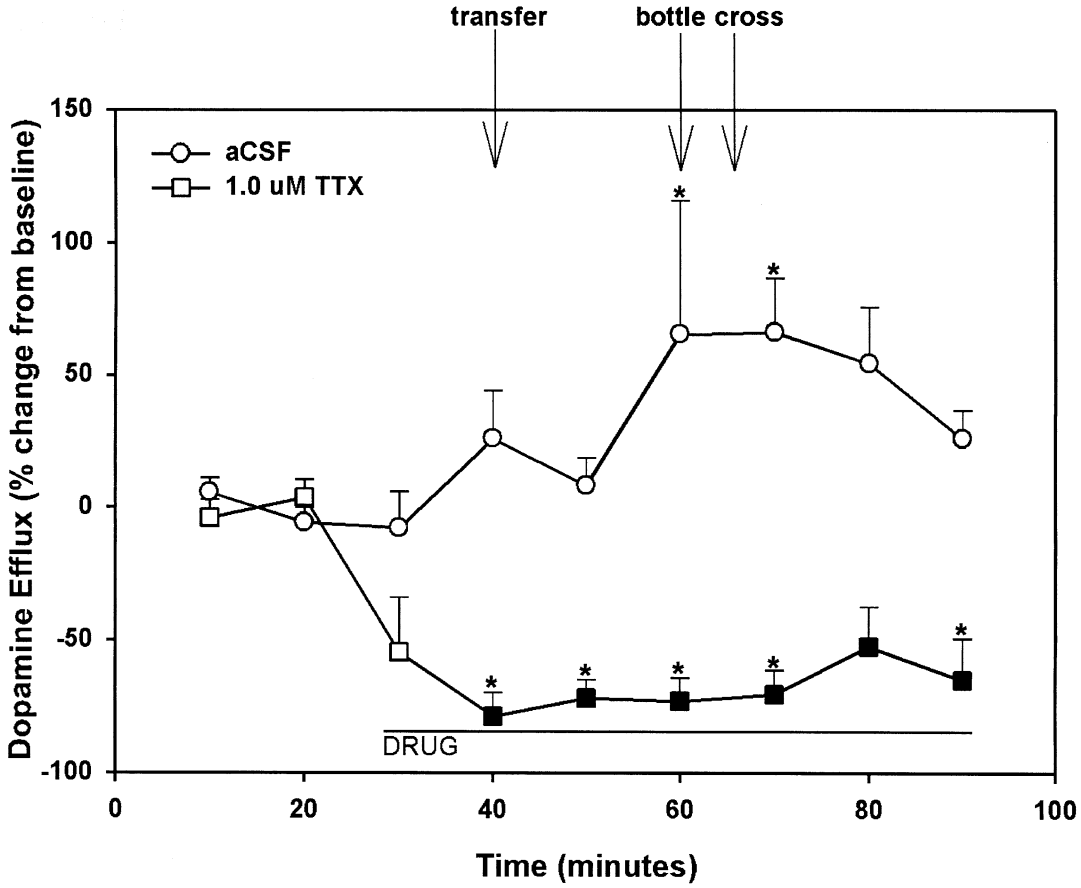

Fig. 3. Effects of NAC perfusion of aCSF (n=6) or TTX (1 μM, n=6) on NAC DA efflux (see legend of Fig. 2 for symbols and other relevant information). In animals perfused with aCSF, NAC DA efflux increased significantly over baseline as the bottle was presented in compartment A, the animals licked the solution, the screen was removed, the animals crossed to compartment B and consumed the palatable food. NAC perfusion of TTX significantly decreased NAC DA efflux from baseline (* $P<0.05$) and attenuated the increases in DA efflux seen in aCSF-perfused rats (■ denotes a significant difference from the aCSF session; $P<0.05$).

RESULTS

Microdialysis probe placement

Fig. 1 depicts representative placements of probes in the mPFC, shell of the NAC and the STR (see legend for more details).

Basal ACh and DA efflux

In animals equipped with NAC probes, mean basal ACh efflux in the mPFC was 0.036±0.004 pmol. Basal ACh efflux did not differ across sessions ($F_{3,15}$=0.93, P=0.45), nor, within session, across the four collections taken prior to drug infusion ($F_{3,15}$=1.11, P=0.38). The stability of basal ACh efflux was further indicated by the absence of a significant interaction between session and these four baseline collections ($F_{9,45}$=1.04, P=0.42). Likewise, basal NAC DA efflux was stable within and across sessions (6.630±0.890 pmol; SESSION: $F_{3,15}$=1.69, P=0.21; TIME: $F_{1,15}$=2.69, P=0.16; SESSION×TIME, $F_{3,15}$=2.81, P=0.08). In control animals with probes placed in the STR, basal ACh again did not vary within or across sessions (0.025±0.002 pmol; (SESSION: $F_{1,6}$=0.03, P=0.86; TIME: $F_{3,18}$=1.343, P=0.29; SESSION×TIME, $F_{3,18}$=0.60, P=0.63). Subsequent expressions of neurotransmitter efflux are expressed as a percent change from the mean (±S.E.M.) basal efflux.

Behavior-associated increases in mPFC ACh efflux, NAC DA efflux, and attenuation by NAC TTX

Overall, ACh efflux in mPFC increased over the course of the various events of the task (Fig. 2; main effect of TIME: $F_{17,85}$=11.47, $P<0.0001$). The increases in ACh efflux differed between animals with intra-accumbens TTX versus aCSF perfusions (DRUG×TIME: $F_{17,85}$=2.04, P=0.02). Multiple comparisons indicated that in animals perfused with aCSF there was an increase in ACh efflux associated with transfer from the baseline bowl to the apparatus. This increase then quickly returned to baseline during ensuing collections. Cortical ACh efflux was again increased during exposure to the bottle and licking in Compartment A and this increase was maintained while the animal gained access to Compartment B and consumed the food reward (see Figure and legend for results from multiple comparisons). In contrast, animals perfused with

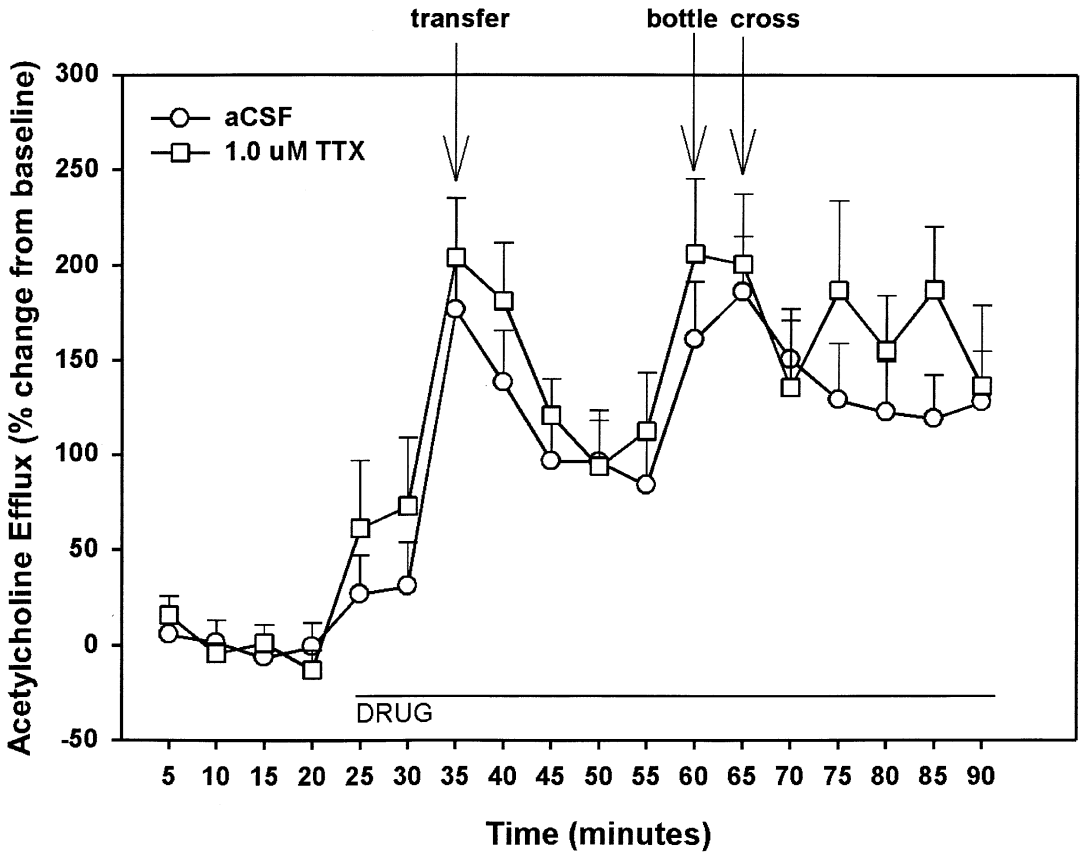

Fig. 4. Effects of STR perfusion of aCSF ($n=7$) or TTX (1 μM, $n=7$) on behavior-associated mPFC ACh efflux. As seen in animals equipped with NAC probes, mPFC ACh efflux increased in association with the animals' transfer into compartment A, presentation of the bottle, licking, screen removal and crossing, and food consumption. Perfusion of TTX (1 μM) into the STR did not affect behavior-associated ACh efflux (see legend of Fig. 2 for more details).

TTX into the NAC exhibited a modest increase over baseline for all collections after transfer, but multiple comparisons did not reveal any significant differences.

Although there was no overall effect of behavioral manipulations on NAC DA efflux (TIME: $F_{8,40}=1.95$, $P=0.08$), there were, importantly, a main effect of DRUG ($F_{1,5}=29.21$, $P=0.003$), and a significant interaction between the effects of DRUG and TIME ($F_{8,40}=6.58$, $P=0.00002$; see Fig. 3). Multiple comparisons indicated that, during aCSF perfusions, NAC DA levels were increased in collections taken after bottle presentation, drinking, screen removal, cross, and food consumption. In contrast, in TTX-perfused animals, such increases were not found and, in fact, NAC DA levels remained well below baseline during the entire behavioral session (see legend for details). Although, once again, these experiments were not designed to determine the behavioral variables that stimulate NAC DA release, it is noteworthy that transfer of the animals from the bowls to compartment A did not significantly affect NAC DA whereas transfer did lead to an increase in mPFC ACh efflux (Fig. 2). Accordingly, correlations between the magnitude of behavior-associated changes in accumbens DA and mPFC ACh efflux were not significant, neither when analyzed over all data pairs nor when selectively analyzed with respect to individual behavioral events (transfer, bottle presentation, food consumption; all $P>0.18$).

Effects of TTX perfusion into the STR

In animals with STR probes, mPFC ACh efflux was again increased in association with the behavioral manipulations (main effect of TIME: $F_{17,102}=24.00$, $P<0.0001$; Fig. 4; see legend for more details). Perfusion of TTX into the STR did not affect cortical ACh efflux ($F_{1,6}=0.97$, $P=0.36$) and there was no interaction between the effects of DRUG and TIME ($F_{17,102}=1.08$, $P=0.39$; see Fig. 4).

Effects of NAC perfusion of SULP

Once again, mPFC ACh efflux was increased by the behavioral manipulations (main effect of TIME: $F_{17,85}=9.90$, $P<0.0001$). Intra-NAC perfusion of drug did not affect mPFC efflux ($F_{2,10}=0.36$, $P=0.71$), and the effects of SULP and the behavioral manipulations did not interact ($F_{34,170}=0.70$, $P=0.89$; see Fig. 5). Likewise, NAC DA efflux increased during and after bottle presentation, drinking, cross, and food consumption ($F_{8,40}=4.71$, $P=0.0004$), but perfusion of SULP did not significantly affect NAC DA efflux (DRUG: $F_{2,10}=1.32$, $P=0.31$; DRUG×TIME: $F_{16,80}=0.75$, $P=0.73$; not shown).

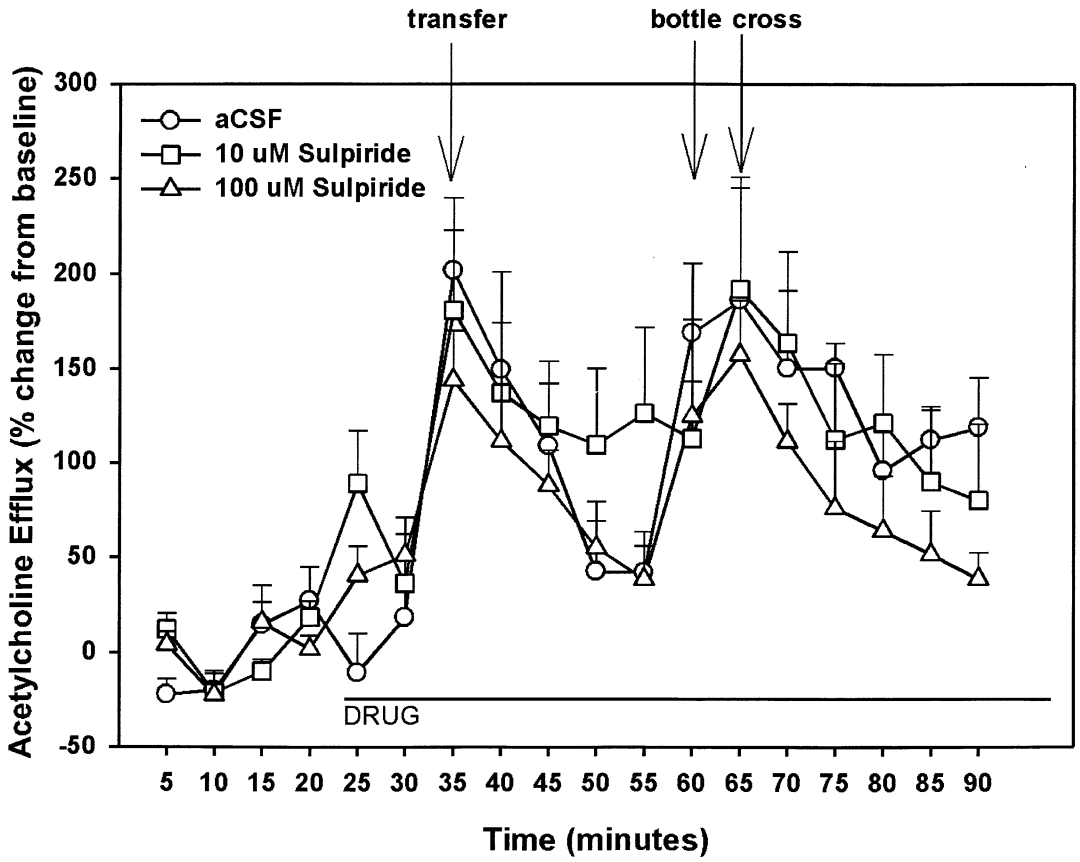

Fig. 5. Effects of intra-NAC perfusion of either aCSF (n=6) or SULP (10 or 100 μM; n=6; see legend of Fig. 2 for symbols and other relevant information). Once again, mPFC ACh efflux was increased in association with the behavioral manipulations and the behavior of the animals. Perfusion of SULP into the NAC did not affect mPFC ACh efflux.

Effects of NAC TTX or SULP on behavioral performance

During infusions of TTX into the NAC, only two of six animals reached the criterion for 500 licks within 5 min before all rats were allowed to cross over into compartment B. Inspection of the microdialysis data revealed highly overlapping values of cortical ACh efflux between rats that reached criterion compared and those that did not. All animals perfused with aCSF reached this criterion and thus were allowed to cross prior to reaching the alternative 5 min criterion. However, NAC perfusion of TTX did not significantly affect the number of licks, the time required to complete the first 100 licks, the latency to contact the food in compartment B, or the time required to consume the food. Likewise, perfusion of SULP or perfusion of TTX into the STR did not affect behavioral performance (all $P>0.05$; see Fig. 6; note the large variability of the effects of NAC TTX on latency to 100 licks).

DISCUSSION

These experiments were designed to test the hypothesis that neuronal activity within the NAC is necessary for behavior-associated increases in cortical ACh release. Three important observations emerged. First, performance in a behavioral task that requires rats to drink a mildly aversive solution in order to gain access to a highly palatable food were associated with increases in the efflux of both NAC DA and cortical ACh. Second, local perfusion of TTX within the shell region of the NAC blocked the increase in NAC DA efflux and, as expected, markedly reduced basal levels of DA efflux. Intra-NAC perfusion of TTX prevented the performance-associated increase of cortical ACh efflux without affecting basal levels. A control perfusion of TTX in the STR failed to affect cortical ACh efflux. Third, the intra-NAC perfusion of the D2 antagonist SULP did not reproduce the TTX effect, indicating that interruption of D2 receptor activity is not a necessary component of the TTX effect. The discussion that follows expands upon these observations by considering the degree of coordination between changes in NAC DA and cortical ACh efflux and their relation to task events, mechanisms by which NAC efferent activity might influence the BFCS, and speculation about the functional implications of a trans-synaptic regulation of basal forebrain excitability by the NAC.

Performance-related changes in NAC DA efflux

Transfer of the animals from the bowls to compartment A produced a modest trend toward an increase in NAC DA efflux; however, this increase did not reach statistical sig-

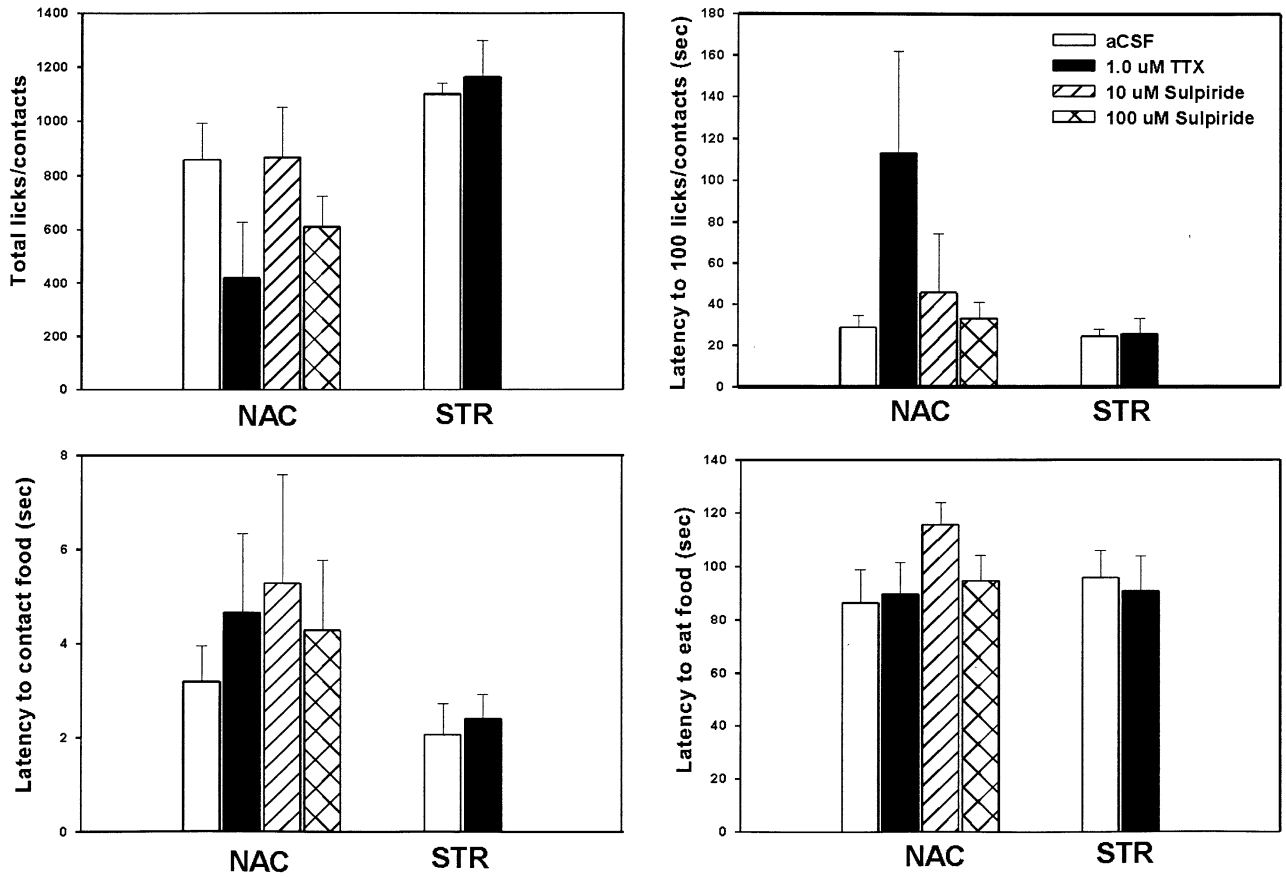

Fig. 6. Effects of drug perfusions into the NAC or STR on behavioral measures (mean+S.E.M.). There were no effects of any drug administration on the number of licks, the latency to completion of the first 100 licks, the latency to contact the food, or the latency to complete consumption of the food (note the large variability of the effects of NAC TTX on the latency to 100 licks).

nificance. The absence of an increase in NAC DA efflux during this part of the task corresponds with previous findings suggesting that NAC DA efflux does not change in animals that are transferred into novel environments (Neigh et al., 2001) or into familiar environments that are associated with appetitive processes (Wilson et al., 1995; Floresco et al., 1996; Bassareo and Di Chiara, 1999). Likewise, the significant increases in NAC DA efflux observed after insertion of the bottle and providing access to the palatable food in the present experiment conforms with a substantial literature that suggests that food consumption per se, and particularly if associated with conditioned incentive properties (as was the case for the licking of the citric acid solution associated with subsequent access to a palatable food) is sufficient to increase NAC DA efflux (Young et al., 1992; Mark et al., 1994; Taber and Fibiger, 1997; Berridge and Robinson, 1998; Sokolowski et al., 1998; Ahn and Phillips, 1999).

Performance-related changes in cortical ACh efflux

Transfer of the animals from the bowls to compartment A resulted in a marked increase in cortical ACh efflux that then steadily declined toward baseline values during the ensuing four collections. The mere act of handling rats has been shown to increase ACh efflux in frontal cortex (Thiel et al., 1998) and this effect does not completely habituate. One might have expected a greater degree of habituation in the present experiments given the extensive handling associated with training; however, the transfer to compartment A is also associated with considerable arousal and expectancies surrounding the presentation of the bottle, removal of the separating screen and opportunity to move into compartment B. This initial increase in cortical ACh efflux returns to the original baseline within 30 min after transfer.

Exposure to the drinking bottle resulted in a rapid elevation of cortical ACh to a level comparable to that seen following the initial transfer. The available literature on the activity of cortical cholinergic inputs associated with complex motivational processes such as those triggered by the sequence of aversive and appetitive stimuli used in the present experiment (bottle presentation, licking citric acid solution, removal of screen, palatable food) is very limited. Acquas and colleagues (1996) have shown that fearful stimuli will increase cortical ACh efflux as will previously neutral stimuli conditioned to predict exposure to that stimulus. While licking the citric acid solution, the animals were also presumably anticipating the opening of the divider,

allowing for the crossing over into compartment B and the opportunity to consume the palatable cheese snack. Previous research has demonstrated that intervals directly preceding the opportunity to ingest palatable foods and fluids are associated with marked increases in cortical ACh efflux (Inglis et al., 1994; Ghiani et al., 1998).

Finally, there is considerable evidence demonstrating a relationship between cortical cholinergic transmission and attentional processing in humans and in laboratory animals (see introduction for reviews). Recent microdialysis studies in rats performing tasks associated with explicit demands on attentional processing reveal marked increases in cortical ACh efflux (Passetti et al., 2000; Dalley et al., 2001; Himmelheber et al., 2001; Arnold et al., 2002). While it seems reasonable to assume that performance in the present complex task might have involved attentional processing, particularly during the periods of transfer, exposure to the bottle and the removal of the partition between the compartments, this task, unlike those cited above, was not designed to explicitly tax attentional capacities.

Relationship between NAC DA and cortical ACh efflux

The goal of the present experiment was to produce behavior-associated increases in NAC DA and cortical ACh efflux and then to test the hypothesis that NAC transmission is necessary for increases in ACh efflux. While the available data support this hypothesis, the relationship between NAC DA and cortical ACh efflux was partial, as indicated by the observation that the increase in cortical ACh efflux during the transfer phase was not paralleled by an increase in NAC DA efflux during this phase. As intra-NAC TTX blocked all behavior-associated increases in cortical ACh efflux, the available data may be interpreted as suggesting that increases in some non-dopaminergic transmission within NAC is necessary for the observed increases in cortical ACh efflux in animals performing this task (see Discussion below). It is also possible, however, that changes in NAC DA efflux, measured by microdialysis, do not adequately reveal the rapid and dynamic NAC dopaminergic mechanisms contributing to this relationship.

Effects of pharmacological manipulations on transmitter efflux and task performance

The local administration of TTX into the shell of the NAC completely prevented any performance-related increases in NAC DA efflux and supports the conclusion that stimulation of DA efflux reflected an increase in impulse-dependent DA release. TTX also resulted in a substantial reduction in basal DA values. These data are consistent with numerous microdialysis studies (for review, see Westerink and Timmerman, 1999) and demonstrate that almost all extrasynaptic DA, harvested by the dialysis probe, is the product of physiologically released DA. Intra-NAC perfusion of TTX also eliminated task-induced increases in cortical ACh efflux demonstrating that the rise in cortical ACh levels reflected impulse-dependent increases in NAC transmission. Unlike the case with basal NAC DA efflux, however, basal cortical ACh was unaffected by perfusion of TTX suggesting that impulse-dependent transmission within NAC is not necessary for the maintenance of basal levels of cortical ACh release. Importantly, perfusion of TTX into the STR had no significant effect on cortical ACh efflux, suggesting that diffusion of the drug outside of the NAC and into the STR was not responsible for the effects on transmitter efflux. This does not preclude the possibility that TTX diffused, in sufficient quantities, to areas outside of the shell (e.g. core region of the NAC) to affect the BFCS. Studies are in progress to evaluate this possibility.

Administration of SULP into the NAC or TTX into the STR had no effect on any of the measures of behavioral performance. Somewhat unexpected, however, was the absence of any significant effect of the intra-NAC infusion of TTX on performance measures. This was in stark contrast to the marked attenuation of DA and ACh efflux. However, the data revealed two interesting trends that, due to high within-group variability, failed to reach statistical significance. The first trend was a TTX-induced decrease in the total number of licks or contacts with the drinking spout, and the second was a TTX-induced increase in the latency to reach 100 licks on the drinking tube. Once again, attempts to attribute these effects to changes in NAC-cortical interactions would require experiments specifically designed to test hypotheses about the role of these interactions in motivated behaviors.

Potential mechanisms underlying the TTX effect

As stated earlier, the literature has revealed an accumbens DA-mediated modulation of basal forebrain excitability (Yang and Mogenson, 1989; Moore et al., 1999). Thus, it is conceivable that the TTX-induced blockade of stimulated ACh efflux reflects the ability of TTX to potently reduce extracellular DA and hence NAC DA receptor activity. The inability of the D2 antagonist SULP to affect cortical ACh efflux is not consistent with a necessary role of the D2 receptor subtype for task-induced increases in cortical ACh release. This is in marked contrast to the ability of D2 antagonists to block pharmacologically induced cortical ACh release (Moore et al., 1999); however, one cannot discount possible differences between the neurochemical regulation of BFCS excitability under conditions of behavioral vs pharmacological activation. It could also be argued that simultaneous blockade of D1 and D2 receptors is necessary for this effect as was demonstrated under other conditions such as ACh release following tactile stimulation (Acquas et al., 1996). Naturally, the potent effect of TTX on extracellular DA would be expected to simultaneously decrease D1 and D2 receptor activity.

At the same time, the effectiveness of TTX may reflect the ability of the drug to simultaneously reduce receptor activity of a number of accumbens transmitter systems, most notably, glutamate (Glu). We have recently demonstrated, that intra-NAC perfusion of NMDA is sufficient to increase prefrontal ACh efflux (Gatien et al., unpublished observations). TTX might interfere with this NMDA-mediated cortical ACh release. However, the relationship between Glu receptor activity and cortical ACh release, at

least under basal conditions, is complex. We have demonstrated that blockade of NMDA and AMPA-kainate receptors *also* increase prefrontal cortical ACh release (Neigh-McCandless et al., 2002). At this stage, we do not understand how Glu receptors within the NAC modulate cortical ACh release under conditions in which the BFCS is activated by a cognitive task.

In summary, to our knowledge, this is the first demonstration of an accumbens-based modulation of cortical ACh release in a task-performing animal. Performance in a task requiring a rat to perform a presumably aversive response in order to gain access to a palatable food was sufficient to stimulate the release of DA in NAC and ACh in mPFC. NAC function has been generally described in terms of the attribution of motivational properties to previously neutral stimuli and contexts, forming the basis for goal-directed behavior maintained by conditioned stimuli (e.g. Whishaw and Kornelsen, 1993). Complex behavioral situations require that the subject search for, detect, select, and discriminate such stimuli from the abundance of available, multimodal stimuli. Such processes have been extensively demonstrated to depend on the activity and integrity of the cortical cholinergic input system (Sarter et al., 2001). Thus, the NAC regulation of cortical ACh efflux is hypothesized to mediate the attentional functions and capacities involved in goal-directed behavior. Furthermore, dysregulation of cortical cholinergic activity may therefore contribute to the mediation of the cognitive impairments of disorders that involve abnormalities in NAC transmission, particularly schizophrenia and compulsive drug addiction (Sarter and Bruno, 1999). While the functional relationship, if any, between NAC transmission and cortical ACh release under conditions of cognitive activation remains to be specified, this type of task provides a platform in which to study the role of the NAC in modulating the excitability of the BFCS in behaving animals.

Acknowledgements—This research was supported by PHS grants MH57436, MH063114 and NS37026.

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(Accepted 9 October 2003)