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Short communication

Dissociations between the effects of intra-accumbens administration of amphetamine and exposure to a novel environment on accumbens dopamine and cortical acetylcholine release

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Abstract

Previous research has demonstrated an interaction between the effects of amphetamine and exposure to a novel environment on the activity of neurons in the nucleus accumbens. Given a model in which these accumbens efferents gate the excitability of basal forebrain cholinergic corticopetal neurons, the administration of intra-accumbens amphetamine was hypothesized to potentiate the increase in cortical acetylcholine produced by introduction to a novel environment. Dual probe microdialysis revealed no synergistic interactions between exposure to a novel environment and amphetamine on nucleus accumbens dopamine or cortical acetylcholine efflux. This finding indicates that exposure to a novel environment failed to recruit the telencephalic activation of the nucleus accumbens presumably necessary to reveal modulatory effects of accumbens dopaminergic transmission on cortical acetylcholine release.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Interactions between neurotransmitters

Keywords: Acetylcholine; Dopamine; Microdialysis; Cortex; Nucleus accumbens; Novel environment

All cortical areas and layers are innervated by cholinergic projections that originate in the basal forebrain/substantia innominata [19]. The basal forebrain cholinergic system serves to gate cortical information processing and, as such, affects attentional processes [18]. Pharmacological modulation of the excitability of the basal forebrain cholinergic neurons affects acetylcholine (ACh) release in the cortex and subsequent attentional processing [17]. Based on earlier work, it has been postulated that enhanced dopaminergic transmission within the nucleus accumbens (NAC) potentiates cortical ACh efflux by inhibiting the GABAergic projections from the NAC to the basal forebrain [11,20]. Systemic administration of amphetamine, an indirect dopamine (DA) agonist, has provided evidence that may indirectly support this model. Systemic amphetamine increases cortical ACh efflux, and this increase can be attenuated by either the systemic administration of D1 or D2 antagonists or forebrain DA depletions [6]. In contrast, intra-accumbens administration of amphetamine, despite producing large increases in NAC DA efflux, was not sufficient to potentiate the increase in cortical ACh efflux elicited by an environmental stimulus [3].

A possible interpretation of this inability of local administration of amphetamine to increase cortical ACh release is that although excitatory inputs to the basal forebrain were transiently activated by the environmental stimulus, there was inadequate activation of NAC afferents, limiting the ability of amphetamine-induced DA release in the NAC to modulate GABAergic efferents [12]. DA has been postulated to have different effects on accumbens activity depending on whether the accumbens projection neurons are in an up-state or a down-state. For example, hippocampal input is necessary for NAC neurons to enter a depolarized and active state (up-state), acting to gate prefrontal-accumbens through-put [13]. If the neurons in the NAC are in an up-state, activation of D1 receptors

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may act to maintain excitatory responses. However, if these neurons are in a down-state, D1 receptor activity is ineffective, and D2 receptor activity may serve to hold the down-state [12]. The systemic administration of amphetamine may result in the appropriate recruitment of the above proposed circuit as it elicits increases in both cortical ACh and NAC DA [3]. Therefore, it can be postulated that the use of a behavioral stimulus that increases the afferent activity to both the basal forebrain cholinergic system and NAC may reveal an effect of intra-accumbens amphetamine on cortical ACh release.

Exposure to novelty is a potent behavioral stimulus that activates the basal forebrain cholinergic system resulting in increases in cortical ACh efflux [1]. These novelty-induced increases in cortical ACh efflux can be blocked by the systemic administration of DA antagonists [2]. Furthermore, DA-related voltammetric signals in the NAC are increased following entrance into a novel environment [16], and more specifically, DA efflux in the NAC has also been shown to significantly increase during exploration of novel stimuli [10]. Finally, exposure to a novel environment has been shown to potentiate the effects of amphetamine. Novelty enhances sensitization to the psychomotor activating effect of amphetamine [7] and facilitates the induction of sensitization to amphetamine at lower doses [5]. A novel environment can also enhance the neurobiological effects of amphetamine, as measured by c-*fos* expression, in the striatum and cortex [4].

Therefore, in the present experiment we determined whether the local administration of amphetamine into the shell of the NAC, in conjunction with exposure to a novel environment, would reveal an ability of the drug to affect cortical ACh release that is not seen following intra-accumbens perfusion of amphetamine in a familiar environment [3]. It was predicted that if exposure to the novel environment sufficiently activated both the NAC and basal forebrain, then intra-accumbens perfusion of amphetamine would negatively modulate NAC efferents and hence potentiate cortical ACh release via disinhibition of basal forebrain cholinergic neurons.

Thirteen adult male Fisher-344/Brown Norway F1 hybrid rats (Harlan, Indianapolis, IN) were used for this study. The animals were housed under a 12:12-h light–dark cycle, with food and water ad libitum. Animal care and use was in accordance with the University Institute Laboratory Animal Care and Use Committee of The Ohio State University and the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Animals were habituated to a familiar environment (standard lighting, opaque concentric dialysis bowl, pine bedding) for 4 days. On the fifth day animals were anesthetized with ketamine (100.0 mg/kg, i.p.) and xylazine (3.0 mg/kg, i.p.), and microdialysis guide cannulas (BAS, West Lafayette, IN) were stereotaxically implanted in the medial prefrontal cortex (mPFC; A=3.5 mm, L=0.8 mm, V=1.0 mm, angled 10° toward anterior) and the ipsilateral shell of the NAC (A=0.2 mm, L=1.0 mm, V=4.9 mm, angled 15° toward posterior) [14]. Both guide cannulae were fixed to the skull with stainless-steel screws and dental acrylic. Hemispheres were counterbalanced across animals.

The first microdialysis session was conducted 3 days following surgery. Animals were placed in the familiar environment for at least 30 min prior to the insertion of concentric microdialysis probes (2.0 mm membrane, BAS) into the mPFC and NAC. Probes were attached to a dual channel swivel (Instech, Plymouth Meeting, PA) and perfused (1.25 μl/min) with an artificial cerebrospinal fluid (aCSF) that contained the following (in mM): NaCl, 166.5; $NaHCO_3$, 27.5; KCl, 2.4; Na_2SO_4, 0.5; $CaCl_2$, 1.2; $MgCl_2$, 0.8; glucose, 1.0. In addition, the perfusate for the mPFC probe contained the acetylcholinesterase inhibitor neostigmine bromide (0.05 μM). DA collection vials contained 5.0 μl of perchloric acid solution (0.05 N), sodium bisulfite (200 μM) and EDTA (1.0 μM) as an antioxidant. After a 3-h discard period, samples were collected at 15-min intervals. At the end of three baseline collections, D-amphetamine sulfate (0 or 250 μM, Sigma, St. Louis, MO) was perfused through the NAC probe. Subjects were then transferred to a novel environment (dark room, clear concentric dialysis bowl (BAS), and cedar bedding) where collections continued for an additional 105 min. The second dialysis session was identical to the first except that animals remained in the familiar environment for the entire session.

Levels of DA (NAC) and ACh (mPFC) were quantified using high performance liquid chromatography with electrochemical detection according to our standard methods [3]. Briefly, ACh and choline were separated by a C-18 carbon polymer column (ESA, Chelmsford, MA) and ACh was hydrolyzed on a post-column reactor and converted to hydrogen peroxide that was detected [15] with a peroxidase-wired glassy carbon electrode [9]. DA was also separated on a C-18 carbon polymer column (ESA) and detected using a dual electrode coulometric detector (ESA). Nissl stain of thin sections (45 μm) was used to confirm probe placements at the conclusion of the study.

Three-way analyses of variance (ANOVA), used to assess the stability of transmitter efflux during baseline collections, did not reveal any significant effects of time, environment, drug group or any interactions for basal DA or ACh values (all Ps$>$0.05). Subsequent environmental and/or drug effects were expressed as percent change from baseline. Two-way ANOVAs were conducted for the remainder of the statistical analyses to assess main effects of drug, time, or environment and their potential interactions.

As shown in the top of Fig. 1, NAC DA efflux was higher during the novelty session than the familiar session in animals perfused with aCSF (ENVIRONMENT, $F_{1,11}$= 8.491, P=0.014). While visual inspection suggests this modest increase to be most evident during the 15 min

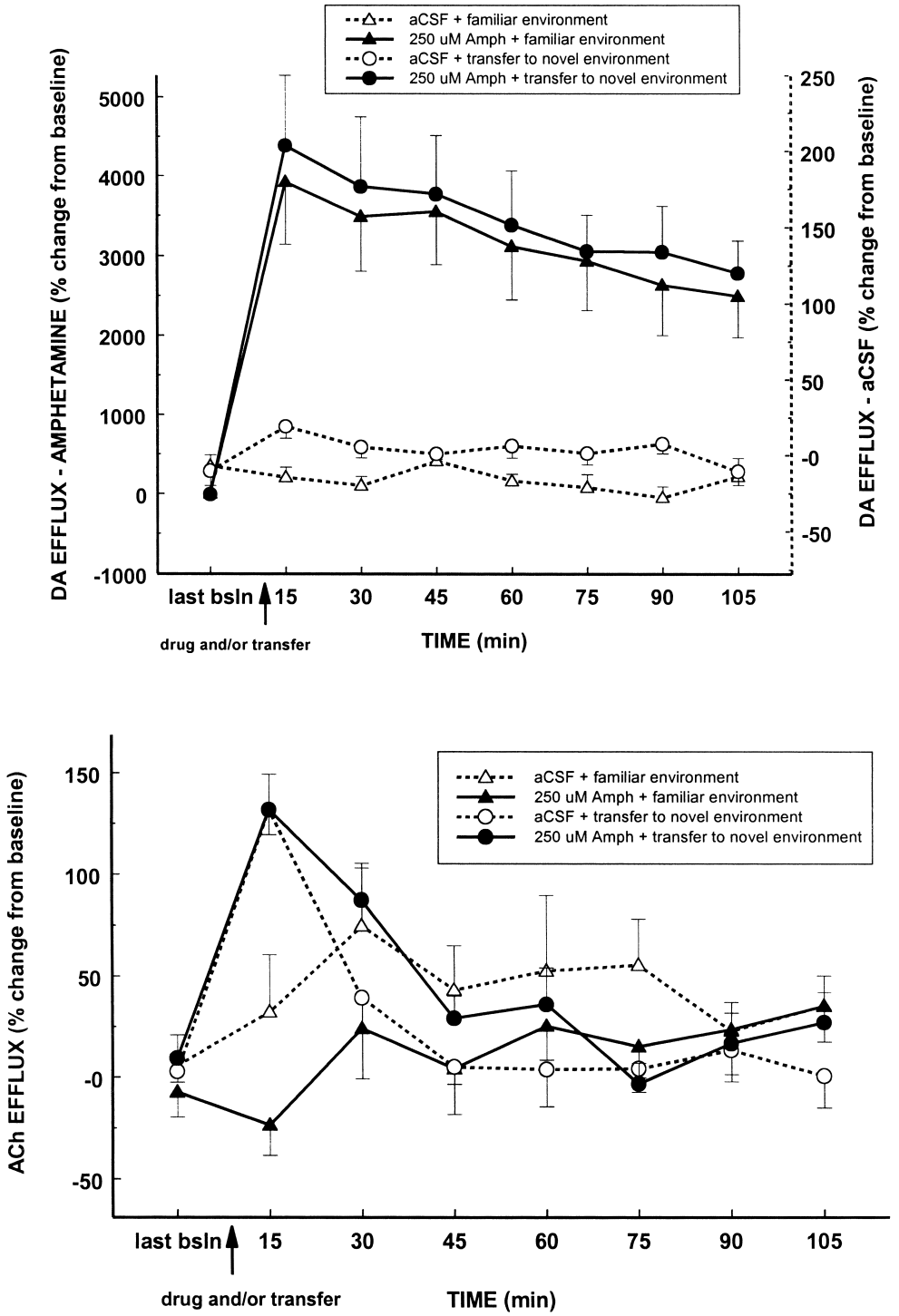

Fig. 1. (Top) Mean (±S.E.M.) level of DA efflux (% change from baseline) in the NAC. 'Last bsln' depicts the mean percent change of the last baseline collection. Control animals received a continuous perfusion of aCSF into the NAC. The perfusion of amphetamine (250 μM) began following the last baseline and continued throughout the session. Following the final baseline, animals were either introduced into a novel environment or remained in a familiar environment for an additional 105 min. Values for % change from baseline are displayed on the left axis for animals that received amphetamine and on the right axis for animals that received aCSF. Simply introducing animals to the novel environment did not result in a significant increase of NAC DA efflux. Although local perfusion of amphetamine significantly increased DA efflux in the shell of the NAC, this effect was similar between the two environments. (Bottom) Mean (±S.E.M.) level of ACh efflux (% change from baseline) in the mPFC. As previously reported [3], intra-accumbens amphetamine had no effect on cortical ACh efflux in a familiar environment. Although introduction into novelty transiently increased cortical ACh, there was no potentiation of this effect by the intra-accumbens perfusion of amphetamine.

following transfer, there were no significant effects of TIME ($F_{7, 77}=0.970$, $P=0.448$) or TIME×ENVIRONMENT ($F_{7, 77}=2.205$, $P=0.061$). As the effects of TIME and ENVIRONMENT did not interact, the main effect of ENVIRONMENT cannot be attributed to the transfer to the novel environment itself. As shown previously [3], perfusion of intra-accumbens amphetamine administration in the familiar environment significantly increased NAC DA efflux (DRUG, $F_{1,11}=43.070$, $P<0.001$; TIME, $F_{7, 77}=30.721$, $P<0.001$; TIME×DRUG, $F_{7, 77}=30.920$, $P<0.001$). Post hoc analyses show that the first collection following amphetamine administration (15 min), and the final collection following amphetamine administration (105 min) were significantly different from the last baseline collection ($t_{12}=-2.43$, $P=0.032$ and $t_{12}=-2.41$, $P=0.033$, respectively). Novelty did *not* potentiate the amphetamine-induced increase in NAC DA efflux (ENVIRONMENT, $F_{1,11}=0.101$, $P=0.757$; TIME, $F_{7, 77}=30.688$, $P<0.001$; TIME×ENVIRONMENT, $F_{7, 77}=0.117$, $P=0.865$).

The bottom panel of Fig. 1 illustrates that transfer to a novel environment did transiently increase cortical ACh efflux. Although ENVIRONMENT did not have an effect ($F_{1,11}=0.613$, $P=0.450$), there was a significant effect of TIME ($F_{7, 77}=3.289$, $P=0.007$), and importantly, a TIME×ENVIRONMENT interaction ($F_{7, 77}=3.208$, $P=0.008$). During the 15 min following the introduction into a novel environment, ACh release was significantly greater in subjects that were transferred into a novel environment than in subjects that remained in the familiar environment ($t_{11}=-2.668$, $P=0.02$). As expected [3], cortical ACh efflux was *not* altered by administration of amphetamine into the NAC without a transfer (DRUG, $F_{1,11}=1.755$, $P=0.212$; TIME, $F_{7, 77}=1.480$, $P=0.199$; DRUG×TIME, $F_{7, 77}=0.605$, $P=0.725$). Furthermore, intra-accumbens administration of amphetamine did not potentiate the novelty induced increase in cortical ACh efflux (DRUG, $F_{1,11}=2.342$, $P=0.154$; TIME, $F_{7, 77}=20.246$, $P<0.001$; TIME×DRUG, $F_{7, 77}=0.931$, $P=0.471$).

The data from this experiment indicate that although intra-accumbens administration of amphetamine potently increased NAC DA efflux, and exposure to a novel environment transiently increased mPFC ACh efflux, there was no interaction between these two manipulations as measured by NAC DA or mPFC ACh efflux. The insufficiency of local increases in NAC DA levels to augment cortical ACh release is consistent with our previous results [3] and does not support the NAC as the sole locus for effects of systemic amphetamine on cortical ACh. However, anatomical [18] and pharmacological evidence [11] suggests that DA can modulate NAC GABAergic afferent projections to the basal forebrain cholinergic system. A possible reconciliation for these observations is that transfer to the novel environment did not sufficiently activate excitatory glutamatergic NAC afferents — a substrate modulated by DA. Consistent with this interpretation is a recent report demonstrating that exposure to a novel environment does not increase glutamate release in the dorsal striatum [8]. As mentioned previously, DA may act as a 'state-stabilizer', in that, if neurons have been driven into an up-state by a contextual stimulus, DA maintains that stimulated state [12]. Perhaps the systemic administration of amphetamine, but not intra-accumbens perfusion, activates the necessary telencephalic circuitry, and therefore results in increased ACh release in the cortex. If introduction to a novel environment did *not* produce sufficient activation to drive NAC neurons into an up-state, local increases in DA release would not sufficiently inhibit the NAC GABAergic projection to the basal forebrain and thus would not be able to potentiate the activity of the basal forebrain cholinergic system. Therefore, the type of exposure to novelty in this report may not have sufficiently activated critical NAC excitatory afferents from several forebrain structures (i.e. cortex, hippocampus, and amygdala).

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