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ARTICLEAmphetamine stimulates movement through  
thalamocortical glutamate releaseOmar S. Mabrouk,<sup>\*,†</sup> Daniel Z. Semaan,<sup>\*</sup> Sarah Mikelman,<sup>†</sup>  
Margaret E. Gnegy<sup>†</sup> and Robert T. Kennedy<sup>\*,†</sup><sup>\*</sup>Department of Chemistry, University of Michigan, Ann Arbor, Michigan, USA<sup>†</sup>Department of Pharmacology, University of Michigan, Ann Arbor, Michigan, USA**Abstract**

The ventrolateral thalamus (VL) is a primary relay point between the basal ganglia and the primary motor cortex (M1). Using dual probe microdialysis and locomotor behavior monitoring, we investigated the contribution of VL input into M1 during amphetamine (AMPH)-stimulated monoamine release and hyperlocomotion in rats. Tetrodotoxin (10  $\mu$ M) perfusion into the VL significantly lowered hyperactivity induced by AMPH (1 mg/kg i.p.). This behavioral response corresponded to reduced cortical glutamate and monoamine release. To determine which glutamate receptors the thalamocortical projections acted upon, we perfused either the  $\alpha$ -amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA)/kainate receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione (NBQX) (10  $\mu$ M) or the N-

methyl-D-aspartic acid (NMDA) receptor antagonist (MK-801) intracortically followed by systemic AMPH. The results show that AMPA/kainate, and to a lesser extent NMDA receptors, mediated the observed effects. As glutamate–monoamine interactions could possibly occur through local or circuit-based mechanisms, we isolated and perfused M1 tissue *ex vivo* to determine the extent of local glutamate–dopamine interactions. Taken together, these results demonstrate that AMPH generates hyperlocomotive states via thalamocortical signaling and that cortical AMPA receptors are an important mediator of these effects.

**Keywords:** amphetamine, dopamine, glutamate, microdialysis, motor cortex, thalamus.

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The cortico-basal ganglia-thalamo-cortical loop is a multisynaptic circuit critical for the execution of motivational and motor commands (Alexander *et al.* 1986; Albin *et al.* 1989). Disruptions to this circuit are evident in Parkinson's disease where striatal dopamine loss triggers an opposite dysregulation of striatofugal pathways, resulting in an overactivation of the substantia nigra pars reticulata/globus pallidus internus (SNr/GPi) and a subsequent overinhibition of the thalamocortical relay (Alexander and Crutcher 1990; DeLong 1990; Gerfen *et al.* 1990). Conversely, L-DOPA-induced dyskinesias are thought to arise from excessive dopamine signaling and disinhibition of thalamocortical projections (Albin 1995). The ventrolateral thalamus (VL) in particular is a primary transmitter of basal ganglia information flow into the primary motor cortex (M1; Strick 1976). Based on its key position within the basal ganglia-thalamo-cortical loop, it is essential to describe how VL projections influence cortical neurochemistry during different states of motor activation.

Thalamocortical projections are a major source of glutamate input into cortical areas (Kharazia and Weinberg 1994). Meanwhile, the cortical mantle is widely innervated by

midbrain and brainstem monoaminergic systems including dopamine, norepinephrine (NE), and serotonin (5-HT; Brown *et al.* 1979; Watabe-Uchida *et al.* 2012). Although the majority of cortical glutamate–monoamine interaction studies to date have emphasized the medial prefrontal cortex, these interactions are likely conserved in M1 since it receives dopamine projections from the VTA (Hosp *et al.* 2011) and substantia nigra pars compacta (Watabe-Uchida *et al.* 2012), 5-HT from the raphe nucleus (Arnsten and Goldman-Rakic 1984) and NE from the locus coeruleus (Arnsten and Goldman-Rakic 1984; Gaspar *et al.* 1991). Despite the

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**Abbreviations used:** ACh, acetylcholine; AMPA,  $\alpha$ -amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid; AMPH, amphetamine; LC-MS, liquid chromatography-mass spectrometry; M1, primary motor cortex; NE, norepinephrine; RM, repeated measure; TTX, tetrodotoxin; VL, ventrolateral thalamus.

strong evidence for interactions between cortical glutamate and monoaminergic substrates (Hondo *et al.* 1994; Takahata and Moghaddam 1998, 2003), the source of glutamate which takes part in those interactions has yet to be clearly defined. This is particularly relevant since glutamate can arise from both neuronal and non-neuronal sources such as astrocytes (Parpura *et al.* 1994).

*d*-Amphetamine (AMPH) potently elevates synaptic concentrations of monoamines by reversing monoamine transporters, inhibiting the intracellular vesicular monoamine transporter (VMAT; Brown *et al.* 2001), and increasing phasic exocytotic release (Daberkow *et al.* 2013), all of which results in characteristic hyperlocomotive behavior and stereotypies (Randrup and Munkvad 1969). Thus, according to the classical model of basal ganglia function, psychostimulant-induced dopamine release at the striatal level should ultimately disinhibit thalamocortical projections, enhance cortical glutamate, and stimulate cortico-spinal projection output to facilitate movement. Indeed, previous microdialysis studies have described AMPH-induced glutamate increases in the prefrontal cortex (Reid *et al.* 1997; Del Arco *et al.* 1998).

This study was undertaken to test the hypothesis that VL glutamatergic inputs into M1 are an essential component of AMPH-induced motor activation. To this end we unilaterally perfused the voltage-dependent Na<sup>+</sup> channel blocker tetrodotoxin (TTX) into VL and gave AMPH systemically while monitoring locomotor behavior and thalamocortical neurochemistry. Moreover, to determine which glutamate receptors were involved in this interaction we intracortically perfused antagonists of  $\alpha$ -amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA)/kainate or *N*-methyl-D-aspartic acid (NMDA) receptors followed by systemic AMPH. Finally, using isolated M1 slices, we were able to determine which of these neurochemical effects were because of local glutamate signaling. Since additional neurochemicals could potentially underlie behavioral responses generated by glutamate–dopamine interactions elicited by AMPH, we employed a novel benzoylation liquid chromatography–mass spectrometry (LC-MS) neurochemical monitoring system (Song *et al.* 2012) to examine release dynamics of glutamate, GABA, acetylcholine (ACh), dopamine, 5HT, and NE simultaneously.

## Materials and methods

### Microdialysis

Adult male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing between 250 and 350 g were used for all experiments. Rats were housed in a temperature and humidity controlled room with 12-h light/dark cycles with food and water available *ad libitum*. Adequate measures were taken to prevent animal pain and discomfort. All animals were treated as approved by the University of Michigan Unit for Laboratory Animal Medicine (ULAM) and in accordance with the National Institute of Health (NIH) Guidelines

for the Care and Use of Laboratory Animals. In addition, all animal experiments were conducted in accordance with Animal Research: Reporting *in vivo* Experiments (ARRIVE) guidelines.

Prior to surgery, rats were anesthetized with an intraperitoneal (i.p.) injection of a ketamine (65 mg/kg) and dexdomitor (0.25 mg/kg) mixture prepared in an isotonic salt solution. Concentrically designed microdialysis probes (1 mm dialyzing length) were then implanted unilaterally into M1 and VL using a Model 963 Ultra Precise stereotaxic instrument with 0.1 mm resolution (David Kopf Instruments, Tujunga, CA, USA). Coordinates used for M1 and the VL from bregma and dura were AP +3.2, ML  $\pm$ 2.8, DV –1.2 and AP –2.3, ML  $\pm$  2.6, DV –6.7, respectively (Paxinos and Watson 2007). Probes were secured to the skull by acrylic dental cement and metallic screws. Following surgery, rats were allowed to recover and experiments were run 24 h after probe implantation. Microdialysis probes were flushed at a flow rate of 1.5  $\mu$ L/min with aCSF (composition in mM: CaCl<sub>2</sub> 1.2; KCl 2.7, NaCl 148 and MgCl<sub>2</sub> 0.85) for 3 h using a Chemyx Fusion 400 syringe pump (Chemyx, Stafford, TX, USA). Perfusion flow rate was then reduced to 1  $\mu$ L/min and samples were collected every 10 min. Baseline values were collected for 60 min and then aCSF was switched to include TTX (in VL) or glutamate receptor antagonists (in M1) for 40 min. Following this 40 min period, systemic AMPH was given. When experiments were completed, animals were killed and then brains were extracted and frozen at –80°C until histology.

### Locomotor behavior analysis

While animals were undergoing microdialysis, Logitech (Apples, Switzerland) webcams were placed above cages as previously described (Mabrouk *et al.* 2011). Webcams were connected via USB port to analysis PC running Matlab 2009 (Mathworks, Natick, MA, USA) software. Using the image acquisition toolbox in Matlab, data were collected via a custom designed motion monitoring program (Mark Dow, University of Oregon). Threshold of motion detection software was set at 150. This threshold limit was selected as not to detect small motions (such as breathing or whisker movement) but only larger motions such as walking around cage, running, rearing, etc. Data were collected every 60 s and then binned into 10 min intervals to correlate with neurochemistry. Data are expressed in terms of absolute locomotor activity as calculated by the detection software.

### Motor cortex slice perfusions

M1 from male Sprague–Dawley rats (274–300 g) was extracted, weighed, and placed in chambers of a Brandel perfusion apparatus (Brandel SF-12, Gaithersburg, MD, USA) onto Whatman GF/B filter disks. The volume of these tissue extracts was approximately 1 mm<sup>3</sup>. The chambers were perfused at 37°C with oxygenated Krebs–Ringer buffer (145 mM NaCl, 2.7 mM KCl, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM CaCl<sub>2</sub>, 1.0 mM MgCl<sub>2</sub> mM glucose, 50  $\mu$ M ascorbic acid, 24.9 mM NaHCO<sub>3</sub>, 50  $\mu$ M pargyline and 50  $\mu$ M tropolone) with or without 1  $\mu$ M 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) or 1  $\mu$ M MK-801 for 60 min at a rate of 0.8 mL/min, prior to the start of collection. Fractions were collected every 1 min for 13 min, and a 2 min bolus of 3  $\mu$ M AMPH was added to the samples at fractions 6 and 7. Based on the dead volume of the system, it was calculated that AMPH would be present in fractions 8 and 9. Eluates (0.8 mL per

fraction) were collected into vials then derivatized (as described below) for dopamine analysis.

### Neurotransmitter analysis using QQQ MS

ACh, glutamate, GABA, dopamine, NE, and 5HT were analyzed using a novel LC-MS method recently described by our laboratory (Song *et al.* 2012). For *ex vivo* experiments, 10  $\mu$ L was withdrawn from the total sample volume and then derivatized in the same way as dialysate samples, however, only dopamine levels were investigated. To 10  $\mu$ L samples, 5  $\mu$ L of 100 mM sodium tetraborate was added to the collected 10  $\mu$ L dialysate sample. Then, 5  $\mu$ L of 2% benzoyl chloride in acetonitrile was added to the mixture to initiate the benzylation reaction. 5  $\mu$ L of a stable  $^{13}\text{C}$  benzoylated isotope internal standard mixture was then added to improve quantitation. Finally, d4-ACh was added as an internal standard for ACh. A Waters nanoAcquity (Waters, Milford, MA, USA) system automatically injected 5  $\mu$ L of the sample onto a Waters HSS T3 reverse phase HPLC column (1  $\times$  100 mm, 1.8  $\mu$ m). Mobile phase A consisted of 10 mM ammonium formate and 0.15% formic acid. Mobile phase B was pure acetonitrile. Analytes were detected by an Agilent 6410 (Agilent, Santa Clara, CA, USA) triple quadrupole mass spectrometer operating in positive mode using dynamic multiple reaction monitoring.

### Data presentation and statistical analysis

All neurochemical data were transformed into percent baseline while behavioral data are expressed as absolute locomotor counts. Statistical analysis was performed by two-way repeated measure (RM) ANOVA using Prism 6 (Graphpad Software Inc, La Jolla, CA, USA). In case ANOVA yielded a significant F score, *post hoc* analysis was performed by contrast analysis to determine group differences. In case a significant treatment or time  $\times$  treatment interaction was found, the sequentially rejective Bonferroni test was used to determine specific differences (i.e. at the single time-point level) between groups. *p* values  $< 0.05$  were considered to be statistically significant.

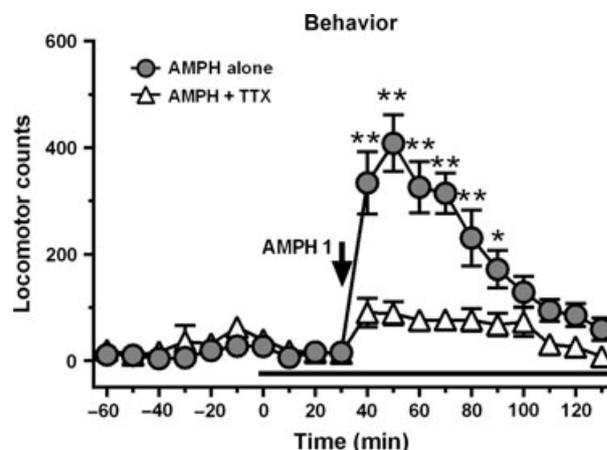
### Materials

AMPH, benzoyl chloride, TTX, NBQX, MK-801, and all LC-MS reagents were purchased from Sigma Chemical Co (St Louis, MO, USA). Ketamine and dexdomitor were from MWI (Rochester Hills, MI, USA). While AMPH was dissolved in isotonic saline solution, NBQX and MK-801 were dissolved in Ringer solution (described above). AMPH was administered by i.p. injections (1 mg/mL/kg) while all other drugs were perfused through the microdialysis probe (reverse dialysis).

## Results

### Effect of TTX perfusion in VL thalamus on AMPH-induced locomotor activity

We first wanted to determine to what extent thalamocortical projections contributed to AMPH-induced hyperlocomotion. To this end, we took advantage of simultaneous locomotor behavior monitoring while perfusing TTX (10  $\mu$ M) directly into the VL via reverse dialysis. Basal locomotor levels were  $22.2 \pm 6.3$  counts/10 min ( $n = 13$ ; Fig. 1). RM ANOVA



**Fig. 1** Systemic amphetamine (AMPH) (1 mg/kg i.p.) administration caused a significant rise in locomotor activity compared to a group which received AMPH (1 mg/kg i.p.) in addition to ventrolateral thalamus (VL) perfusion of tetrodotoxin (TTX) (10  $\mu$ M; black bar). Data are expressed as locomotor counts  $\pm$  SEM. \**p*  $< 0.05$ , AMPH-only time point significantly different from AMPH + TTX treated group. \*\**p*  $< 0.01$ , AMPH-only time point significantly different from AMPH + TTX treated group.

on locomotor activity revealed a significant effect of treatment ( $F_{1,11} = 37.75$ ,  $p < 0.0001$ ), time ( $F_{19,209} = 26.35$ ,  $p < 0.0001$ ), and a time  $\times$  treatment interaction ( $F_{19,209} = 12.56$ ,  $p < 0.0001$ ). Local perfusion of TTX (10  $\mu$ M) had no effect on basal levels of locomotor activity. However, because of low resting activity of animal subjects, our locomotor behavior assay is limited in detecting inhibition of basal motor activity. Nevertheless, AMPH (1 mg/kg i.p.) caused a very large and reliable increase in locomotor activity (maximal  $\sim 408$  locomotor counts 20 min post injection). This effect was largely attenuated by VL perfusion of TTX (Fig. 1).

### Effect of TTX perfusion in the VL thalamus on local AMPH-induced neurochemical changes

The thalamus receives a variety of neurochemical inputs including glutamate, ACh, GABA, dopamine, 5-HT and NE from cortical, subcortical, and brainstem nuclei (Sherman and Guillery 2001). Therefore, to unravel which neurochemicals might be responsible for behavioral modifications induced by AMPH, these neurochemicals were monitored simultaneously. All basal dialysate concentrations from the VL are reported in Table 1. RM ANOVA on AMPH-induced thalamic ACh release showed a significant effect of treatment ( $F_{1,13} = 10.06$ ,  $p = 0.0074$ ), time ( $F_{19,247} = 2.05$ ,  $p = 0.0069$ ), and a time  $\times$  treatment interaction ( $F_{19,247} = 2.93$ ,  $p < 0.0001$ ). AMPH 1 mg/kg i.p. stimulated thalamic ACh release (maximal 96% above baseline; Fig. 2a). TTX (1  $\mu$ M) perfusion through the microdialysis probe prevented this effect and caused a  $\sim 30\%$  reduction in basal values.

Systemic AMPH treatment did not increase VL glutamate or GABA (Fig. 2b and c); however, for GABA, RM ANOVA showed an effect of TTX perfusion ( $F_{1,13} = 34.2$ ,  $p < 0.0001$ ), of time ( $F_{19,247} = 1.14$ ,  $p < 0.0001$ ) and a time  $\times$  treatment interaction ( $F_{19,247} = 6.04$ ,  $p < 0.0001$ ). TTX perfusion also decreased GABA levels by  $\sim 30\%$  (Fig. 2c). TTX reduced dopamine release (maximal  $\sim 60\%$ ) while AMPH stimulated it ( $\sim 700\%$  of baseline values), as expected (Fig. 2d). For dopamine, RM ANOVA showed an

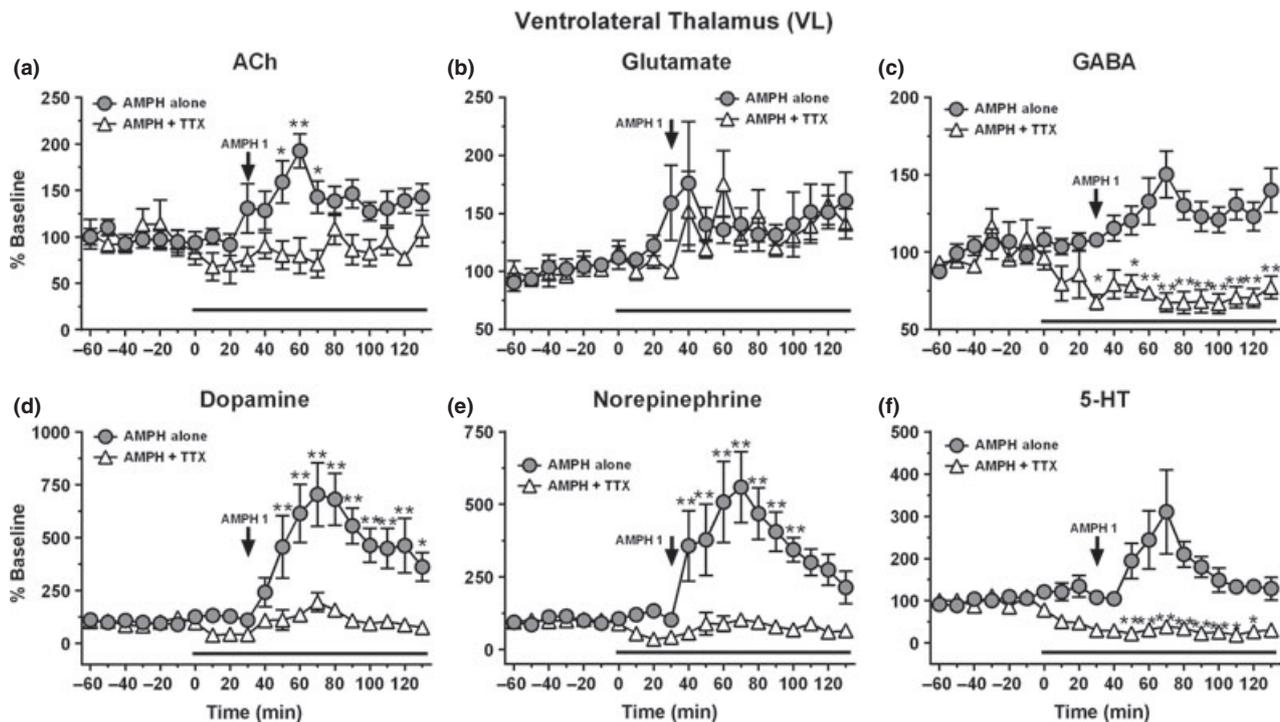
**Table 1** Basal dialysate concentrations of all neurotransmitters analyzed by LC-MS in either the primary motor cortex (M1) or the ventrolateral thalamus (VL). All concentrations are expressed as nanomolar  $\pm$  SEM

Neurotransmitter	Primary motor cortex			Ventrolateral thalamus		
	nM	SEM	n	nM	SEM	n
ACh	21.2	$\pm 1.93$	27	2.5	$\pm 0.28$	25
Glutamate	2459	$\pm 425$	26	214.9	$\pm 24.7$	24
GABA	28.4	$\pm 4.85$	27	19.5	$\pm 2.58$	25
5-HT	0.60	$\pm 0.18$	28	0.43	$\pm 0.16$	26
Norepinephrine	0.91	$\pm 0.17$	26	0.74	$\pm 0.10$	23
Dopamine	0.24	$\pm 0.06$	27	0.28	$\pm 0.09$	25

effect of treatment ( $F_{1,13} = 9.11$ ,  $p = 0.0008$ ), of time ( $F_{19,247} = 10.22$ ,  $p < 0.0001$ ) and a time  $\times$  treatment interaction ( $F_{19,247} = 6.68$ ,  $p < 0.0001$ ). Similar to dopamine, TTX reduced NE levels (maximal  $\sim 60\%$ ) while AMPH stimulated them (maximal  $\sim 560\%$ ; Fig. 5e). For NE, RM ANOVA showed an effect of treatment ( $F_{1,11} = 15.55$ ,  $p = 0.0023$ ), of time ( $F_{19,209} = 6.70$ ,  $p < 0.0001$ ) and a time  $\times$  treatment interaction ( $F_{19,209} = 6.41$ ,  $p < 0.0001$ ). Finally, 5-HT showed a similar effect as the other monoamines with TTX reducing levels (nearly  $\sim 80\%$ ) and AMPH increasing ( $\sim 300\%$ ) with respect to baseline. For 5-HT, RM ANOVA showed an effect of treatment ( $F_{1,12} = 27.29$ ,  $p = 0.0002$ ), of time ( $F_{19,228} = 2.65$ ,  $p = 0.0003$ ) and a time  $\times$  treatment interaction ( $F_{19,228} = 6.91$ ,  $p < 0.0001$ ).

#### Effect of TTX perfusion in the VL thalamus on AMPH-induced neurochemical changes in the M1 motor cortex

The M1 receives a dense glutamatergic projection from the thalamus (Grofová and Rinvik 1974; Herkenham 1980; McFarland and Haber 2002; Hooks *et al.* 2013). To determine the contribution of this circuit to AMPH-stimulated hyperactivation and neurochemical changes, we performed simultaneous dual probe microdialysis in M1 and VL. All basal dialysate concentrations from the M1 are reported in



**Fig. 2** Systemic amphetamine (AMPH) (1 mg/kg i.p.) administration increased thalamic acetylcholine (ACh) (a), dopamine (d), norepinephrine (e), and serotonin (f). These effects were blocked by thalamic perfusion of tetrodotoxin (TTX) (10  $\mu$ M; black bar). AMPH had no effect on VL glutamate (b) or GABA (c). TTX perfusion in VL had no effect on

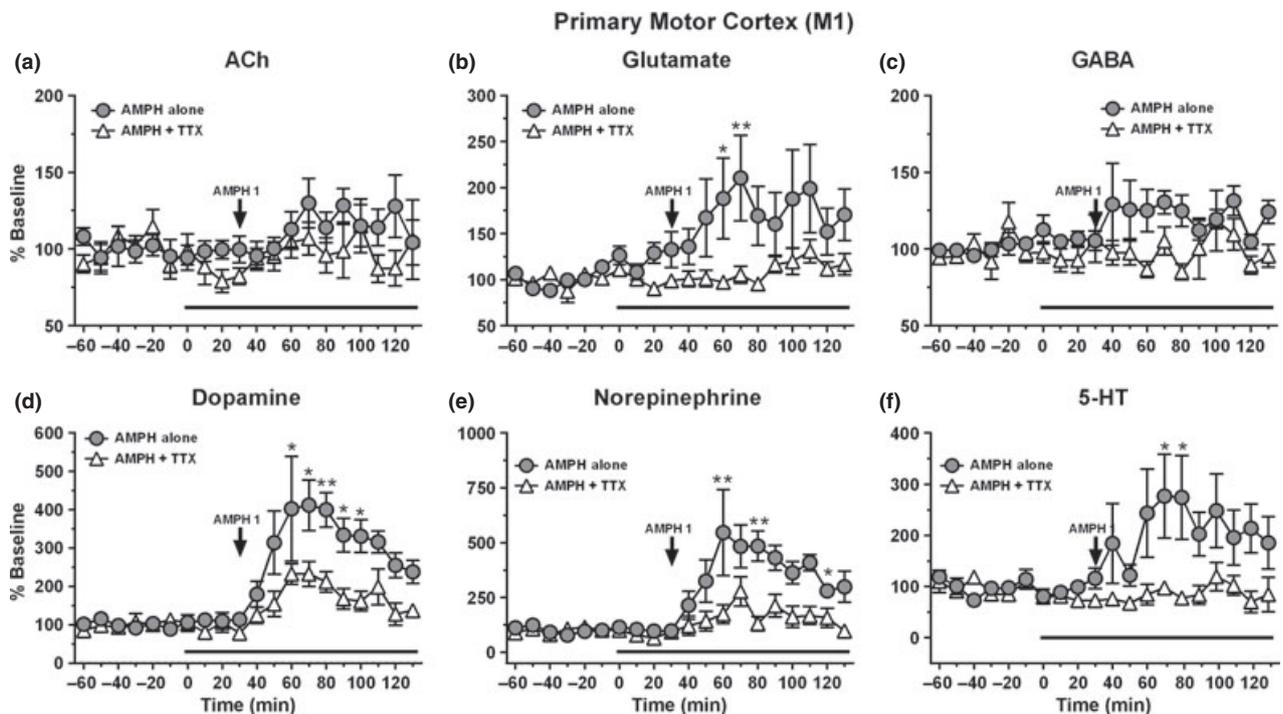
basal glutamate levels (b) but it strongly inhibited GABA (c). Data are expressed as percent baseline  $\pm$  SEM. \* $p < 0.05$ , AMPH-only time point significantly different from AMPH + TTX treated group. \*\* $p < 0.01$ , AMPH-only time point significantly different from AMPH + TTX treated group.

Table 1. Neither thalamic TTX perfusion nor systemic AMPH treatment affected ACh values in M1. AMPH however stimulated the release of cortical glutamate (maximal ~ 210%) while VL TTX perfusion prevented this effect (Fig. 3b). For glutamate, RM ANOVA did not show a significant effect of treatment ( $F_{1,11} = 4.42$ ,  $p = 0.0594$ ), but an effect of time ( $F_{19,209} = 4.23$ ,  $p < 0.0001$ ) and a significant time  $\times$  treatment interaction ( $F_{19,209} = 2.55$ ,  $p < 0.0001$ ). AMPH 1 mg/kg i.p. modestly stimulated cortical GABA release (maximal ~ 30% above baseline) and TTX (1  $\mu$ M) perfusion through the microdialysis probe in the VL prevented this effect (Fig. 3c). For GABA, RM ANOVA showed an effect of treatment ( $F_{1,13} = 6.27$ ,  $p = 0.02$ ), but not time nor a time  $\times$  treatment interaction. As expected, AMPH stimulated the release of dopamine (maximal ~ 410%) at the level of M1 while simultaneous perfusion of TTX (1  $\mu$ M) into the VL largely attenuated this effect (Fig. 3d). For dopamine, RM ANOVA showed an effect of treatment ( $F_{1,13} = 8.48$ ,  $p = 0.012$ ), of time ( $F_{19,247} = 12.80$ ,  $p < 0.0001$ ) and a time  $\times$  treatment interaction ( $F_{19,247} = 2.61$ ,  $p = 0.0004$ ). Similar to dopamine, AMPH stimulated the release of NE (maximal ~ 550%) at the level of the motor cortex while simultaneous perfusion of TTX (1  $\mu$ M) into the VL largely attenuated this effect (Fig. 3e). For NE, RM ANOVA showed an effect of treatment ( $F_{1,11} = 8.96$ ,  $p = 0.012$ ), of time ( $F_{19,209} = 9.26$ ,

$p < 0.0001$ ) and a time  $\times$  treatment interaction ( $F_{19,209} = 3.42$ ,  $p < 0.0001$ ). Finally, 5-HT showed a similar, but more modest effect compared to the other monoamines with AMPH stimulating cortical release (maximal ~ 275%) and VL TTX perfusion attenuating these effects (Fig. 3f). For 5-HT, RM ANOVA showed an effect of treatment ( $F_{1,12} = 6.59$ ,  $p = 0.024$ ), of time ( $F_{19,228} = 1.91$ ,  $p = 0.014$ ) and a time  $\times$  treatment interaction ( $F_{19,228} = 1.87$ ,  $p = 0.018$ ).

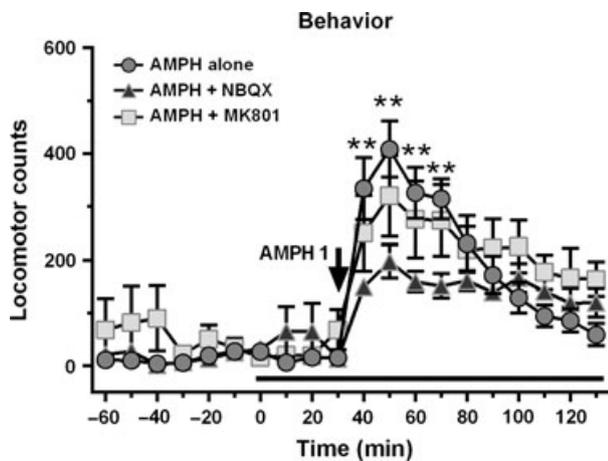
#### Effect of ionotropic glutamate receptor antagonist perfusion in M1 motor cortex on AMPH-induced locomotor activity

Based on our finding that VL activity strongly affected AMPH-induced hyperlocomotion and neurochemistry, we wanted to determine if these effects were mediated through ionotropic glutamate receptors in M1. To this end, we perfused either AMPA (NBQX) or NMDA (MK-801) receptor antagonists in M1 and then treated subjects with AMPH (1 mg/kg i.p.). Basal locomotor levels were  $29.2 \pm 10.6$  counts/10 min ( $n = 18$ ; Fig. 4). RM ANOVA on locomotor activity did not reveal a significant effect of treatment, but an effect of time ( $F_{19,285} = 29.72$ ,  $p < 0.0001$ ) and a time  $\times$  treatment interaction ( $F_{38,285} = 2.30$ ,  $p < 0.0001$ ). Cortical perfusion of either NBQX (10  $\mu$ M) or MK-801 (10  $\mu$ M) had no effect on basal levels of locomotor activity while AMPH stimulated it (maximal



**Fig. 3** Systemic amphetamine (AMPH) (1 mg/kg i.p.) administration increased cortical glutamate (b), dopamine (d), norepinephrine (e), and serotonin (f). These effects were attenuated by thalamic perfusion of tetrodotoxin (TTX) (10  $\mu$ M; black bar). AMPH had no effect on ACh (a)

or GABA (c) in M1. Data are expressed as percent baseline  $\pm$  SEM. \* $p < 0.05$ , AMPH-only time point significantly different from AMPH + TTX treated group. \*\* $p < 0.01$ , AMPH-only time point significantly different from AMPH + TTX treated group.



**Fig. 4** Systemic amphetamine (AMPH) (1 mg/kg i.p.) administration caused a significant rise in locomotor activity. Perfusion of the  $\alpha$ -amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) receptor antagonist NBQX (10  $\mu$ M) in M1 motor cortex attenuated these effects while perfusion of the NMDA receptor antagonist (10  $\mu$ M) was without effect. Data are expressed as locomotor counts  $\pm$  SEM. \*\* $p < 0.01$ , AMPH alone group significantly different from NBQX + AMPH group.

~ 400 counts). The effect of AMPH-induced hyperactivation was partially attenuated by local M1 cortex perfusion of NBQX, but not MK-801 (Fig. 4).

#### Effect of ionotropic glutamate receptor antagonist perfusion in M1 motor cortex on local AMPH-induced neurochemical changes

No difference was seen on ACh or glutamate levels during antagonist perfusion or AMPH treatments (although a trend for AMPH to increase glutamate was observed; Fig. 5b). AMPH 1 mg/kg i.p. modestly stimulated cortical GABA release (maximal ~ 45% above baseline) and NBQX, and to a lesser extent MK-801, prevented this increase (Fig. 5c). For GABA, RM ANOVA showed an effect of treatment ( $F_{2,16} = 12.85$ ,  $p = 0.0005$ ), not time, but a time  $\times$  treatment interaction ( $F_{38,304} = 1.45$ ,  $p = 0.048$ ). MK-801 (10  $\mu$ M) perfusion into M1 enhanced basal dopamine levels. As expected, AMPH stimulated the release of dopamine while local perfusion of NBQX (10  $\mu$ M), and to a lesser extent MK-801 (10  $\mu$ M) attenuated this effect (Fig. 5d). For dopamine, RM ANOVA showed an effect of treatment ( $F_{2,17} = 4.41$ ,  $p = 0.029$ ), of time ( $F_{19,323} = 15.03$ ,  $p < 0.0001$ ) and a time  $\times$  treatment interaction ( $F_{38,323} = 2.99$ ,  $p < 0.0001$ ). Neither antagonist had an effect on basal cortical NE release. Similar to dopamine, AMPH stimulated the release of cortical NE, but differently, NBQX and MK-801 entirely prevented this effect (Fig. 5e). For NE, RM ANOVA showed an effect of treatment ( $F_{2,16} = 6.99$ ,  $p = 0.007$ ), of time ( $F_{19,304} = 8.92$ ,  $p < 0.0001$ ) and a time  $\times$  treatment interaction ( $F_{38,304} = 3.63$ ,  $p < 0.0001$ ). Finally, 5-HT showed a similar effect as the other monoamines with AMPH stimulating cortical release, and NBQX perfusion in M1 strongly attenuated

these effects while the effect of MK-801 was far less pronounced. For 5-HT, RM ANOVA showed an effect of treatment ( $F_{2,16} = 4.83$ ,  $p = 0.023$ ), of time ( $F_{19,304} = 2.08$ ,  $p = 0.006$ ) and a time  $\times$  treatment interaction ( $F_{38,304} = 1.73$ ,  $p = 0.007$ ).

#### Effect of ionotropic glutamate receptor antagonist perfusion in M1 motor cortex on AMPH-induced neurochemical changes in M1 motor cortex slice preparations

As our results pointed towards a complex neurochemical interaction which could be due to either local or circuit-driven processes, we isolated M1 *ex vivo* to examine the contribution of local interactions. We chose to focus on dopamine release in response to local AMPH suprafusion of M1 tissue with or without concomitant suprafusion of ionotropic glutamate receptor antagonists. As expected, AMPH (3  $\mu$ M) stimulated dopamine release from M1 slices (maximal 183% above baseline; Fig. 6). Perfusion of either NBQX (1  $\mu$ M) or MK-801 (1  $\mu$ M) into the M1 cortex attenuated the dopamine releasing effects of AMPH in M1 slices (Fig. 6). For dopamine, RM ANOVA did not show an effect of treatment, but did show an effect of time ( $F_{12,156} = 14.42$ ,  $p < 0.0001$ ) and a time  $\times$  treatment interaction ( $F_{24,156} = 2.44$ ,  $p = 0.0006$ ).

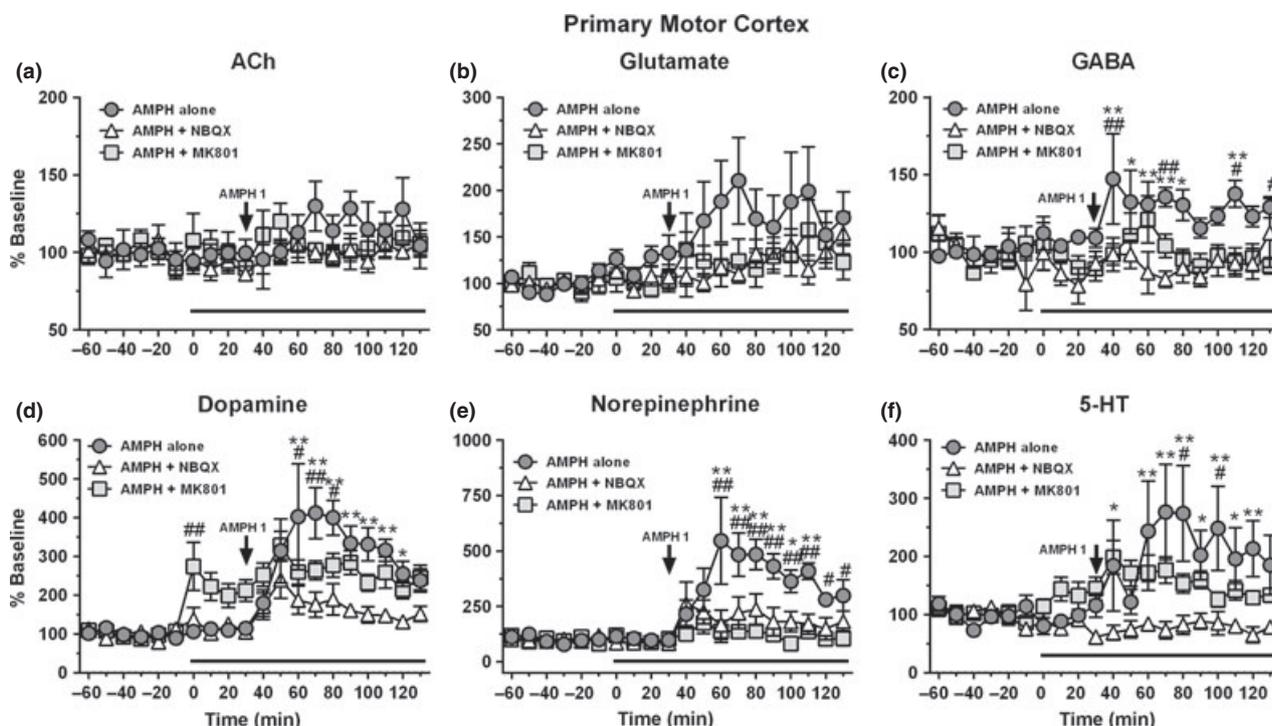
## Discussion

The present study describes a mechanism by which systemic AMPH regulates cortical neurochemistry and motor function, through thalamocortical glutamate projections. As expected, systemic AMPH treatment stimulated hyperlocomotion which corresponded to cortical monoamine and glutamate release. These effects were largely attenuated by concomitant TTX perfusion in the ipsilateral VL or intracortical glutamate antagonist perfusion, suggesting that VL glutamate input into M1 contributes to AMPH-induced movement. Considering the larger inhibitory effect of NBQX with respect to MK-801, glutamate influence appears to be mediated by AMPA receptors and to a lesser extent, NMDA receptors.

#### AMPH-induced behavior and thalamocortical neurochemistry are sensitive to VL thalamus perfusion of TTX

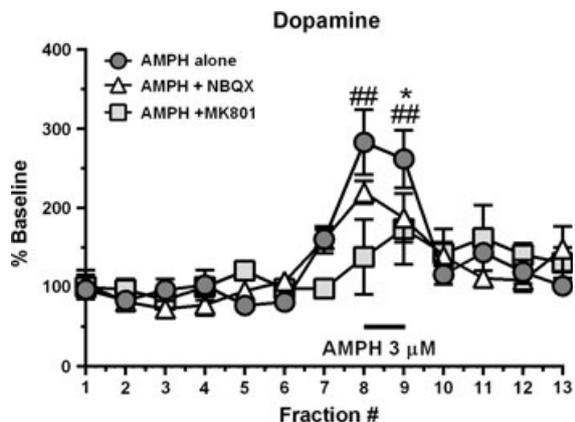
On the basis of its key position between the basal ganglia and the cortex, we asked how heightened monoamine levels (via systemic AMPH treatment) affected thalamic neurochemistry and to what extent this nucleus drives cortical neurotransmission and movement. These results demonstrate that AMPH-mediated movement is processed extrastrially through thalamocortical glutamate transmission.

TTX is a selective voltage-dependent sodium channel blocker, and is commonly used to disclose effects solely due



**Fig. 5** Systemic amphetamine (AMPH) (1 mg/kg i.p.) administration increased M1 GABA (c), dopamine (d), norepinephrine (NE) (e), and 5-HT (f). M1 perfusion of NBQX (10  $\mu$ M; white triangles) blocked AMPH-induced release of GABA, dopamine, NE, and 5-HT. M1 perfusion of MK-801 (10  $\mu$ M) increased dopamine (d), while attenuating AMPH-stimulated dopamine and NE. Antagonist perfusion is represented by the black bar. No differences were observed in ACh (a) or glutamate (b)

levels between any of the groups tested. Data are expressed as percent baseline  $\pm$  SEM. \* $p < 0.05$ , AMPH alone group significantly different from NBQX + AMPH group. \*\* $p < 0.01$ , AMPH alone group significantly different from NBQX + AMPH group. # $p < 0.05$ , AMPH alone group significantly different from MK801 + AMPH group. ## $p < 0.01$ , AMPH alone group significantly different from MK801 + AMPH group.



**Fig. 6** Isolated *ex vivo* M1 slices perfused with amphetamine (AMPH) (3  $\mu$ M; black bar) caused a threefold increase in dopamine levels. Co-perfusion of MK-801 (1  $\mu$ M) and NBQX (1  $\mu$ M) attenuated this increase. Data are expressed as percent baseline  $\pm$  SEM. \* $p < 0.05$ , NBQX-treated group significantly different from AMPH alone group. ## $p < 0.01$ , MK-801-treated group significantly different from AMPH alone group.

to neuronal activity. TTX perfusion effectively ‘shuts down’ the targeted nucleus by preventing action potentials and both tonic and phasic impulse-dependent release events

(Narahashi 1974; Westerink *et al.* 1987). We did not observe any changes in resting locomotor activity during TTX perfusion in VL, but did see a robust attenuation of AMPH-induced hyperactivity, suggesting that AMPH-induced hyperactivation is derived from thalamic activation. As expected and previously reported outside the thalamus, local TTX perfusion in the VL significantly reduced levels of all extracellular monoamines (Westerink *et al.* 1987; Benwell *et al.* 1993), GABA and ACh (Osborne *et al.* 1990; Williams *et al.* 1994; Douglas *et al.* 2001). On the other hand, TTX perfusion did not affect glutamate levels; this is likely because of the ability of non-neuronally derived glutamate sources such as astrocytes to maintain homeostatic extracellular levels of this transmitter (Timmerman and Westerink 1997). AMPH stimulated the release of all three monoamines measured (dopamine, 5HT and NE) both in the VL and in M1 while thalamic TTX perfusion nearly abolished these effects locally in VL. This suggests that AMPH-induced release of monoamines is largely dependent on normal neuronal firing (i.e. impulse dependent release) of monoamine efferents projecting to the thalamus. These findings are in line with recent works which showed that systemic AMPH treatment in behaving or anesthetized

animals produced a phasic, exocytotic-driven release of dopamine at the striatal level (Avelar *et al.* 2013; Daberkow *et al.* 2013).

In addition, systemic AMPH stimulated the release of ACh in the VL to a similar degree as previously reported in the mediodorsal thalamus (Rada *et al.* 2007). This release was TTX-dependent like that observed for monoamine release in this area. The role of thalamic ACh is generally associated with wakefulness (Williams *et al.* 1994) and these results are in line with the known wake-promoting effects of AMPH (Engber *et al.* 1998). The precise mechanism by which ACh is released by AMPH in this area remains a matter of speculation. However, others have shown in hippocampal and striatal areas that AMPH, indirectly acting through DA D1-like receptors elevates extracellular levels (Imperato *et al.* 1993). Since DA in the current experiment was strongly reduced by TTX perfusion, it suggests that DA transmission positively modulates VL ACh.

A main finding of this study was that inhibition of the VL via TTX perfusion significantly attenuated cortical monoamine release. Although this was initially quite surprising, it suggests that glutamate potently modulates cortical monoamine signaling. Indeed, glutamate antagonists delivered directly to the cortex also attenuated AMPH-induced monoamine levels. This type of glutamate modulation has been shown for dopamine in both cortical and subcortical brain structures (Shimizu *et al.* 1990; Wang 1991; Takahata and Moghaddam 1998). Our findings show for the first time that VL glutamate input is responsible for this interaction in M1 and that psychostimulant-evoked monoamine release and movement can be attenuated by cortical glutamate receptor blockade. It is interesting to note that others have shown AMPH-mediated motor behaviors to be strongly attenuated by *systemic* administration of glutamate antagonists (Karler *et al.* 1991, 1995; Kelley and Throne 1992). Our findings point to inhibition of thalamocortical glutamate signaling as the primary mediator of those effects. We also extend the findings of glutamate control over cortical dopamine release to both 5-HT and NE in M1.

#### Cortical perfusion of glutamate antagonists generates a similar behavioral and neurochemical profile as thalamic TTX perfusion

M1 is strongly innervated by glutamatergic projections originating in the VL and VA (for review see Parent and Hazrati 1995). Here, we show that these projections are necessary for AMPH-stimulated hyperactivity and cortical monoamine release. We sought to determine which glutamate receptor subtypes might be responsible for these effects. NBQX perfusion in M1 attenuated AMPH-stimulated hyperactivity while MK-801 produced only a trend to decrease hyperactivity. This was a clue that TTX-induced effects were mediated via cortical AMPA/kainate receptors. However, our neurochemical findings suggested a more complex interac-

tion than a simple glutamate-AMPA receptor mediation of monoamine function. Perfusion of either antagonist did not reduce basal glutamate levels or significantly prevent the rise in cortical glutamate release following AMPH treatment, suggesting that these receptors are not positioned on thalamocortical terminals.

MK-801 perfusion in itself enhanced dopamine levels but did not affect 5-HT or NE. This is in line with previous works in the medial prefrontal cortex (mPFC) which showed that local MK-801 (10  $\mu$ M) perfusion enhanced cortical dopamine 2-fold (Hondo *et al.* 1994) and a more recent work has described cortical activation by NMDA receptor blockade as occurring through the inhibition of cortical GABAergic interneurons (Homayoun and Moghaddam 2007). In this study MK-801 did not significantly reduce GABA levels in itself (although a trend was observed) but did prevent AMPH-induced GABA release, suggesting that NMDA receptor blockade modulates stimulated, but not basal GABA. However, this reduction in stimulated GABA corresponded to reductions in AMPH-induced dopamine and NE release; therefore, apart from increasing tonic dopamine levels through cortical disinhibition, MK-801 may also inhibit phasic dopamine release via receptor blockade at dopamine terminals. In fact, there is strong evidence for NMDA receptor localization to dopamine terminals (Roberts and Sharif 1978; Krebs *et al.* 1991; Desce *et al.* 1992). Therefore, the effects of MK-801 occur through a local cortical mechanism since our *ex vivo* experiments showed that MK-801 perfusion increased dopamine in isolated M1 tissue (data not shown) but also partially prevented AMPH-stimulated dopamine release, similar to our *in vivo* results. It is interesting to note that while MK-801 did not greatly affect AMPH-stimulated 5-HT release, it completely abolished AMPH-stimulated NE release. This strongly suggests that cortical NMDA receptors have diverse pre- and post-synaptic localization on different cell types to selectively modulate cortical neurochemistry.

In contrast to MK-801, NBQX perfusion had no effect on basal dopamine levels and almost completely blocked AMPH-induced dopamine, NE and 5-HT and GABA release. Therefore, similar to our reported behavioral data, AMPA receptor blockade was efficient at preventing AMPH-induced effects. Since NBQX itself did not increase dopamine levels, we can conclude that disinhibition of mesocortical neurons was not involved. However, there is also evidence for pre-synaptic localization of AMPA receptors (Schenk and Matteoli 2004) which may contribute to the observed effect. This is in line with a previous work that showed that cortical AMPA receptor blockade effectively attenuated cortical dopamine release induced by a mild stressor (Takahata and Moghaddam 1998). Therefore, if all monoamine terminals expressed AMPA receptors, NBQX might block phasic release in response to AMPH treatment. However, this idea is not entirely supported by our *ex vivo* data which showed

only a partial attenuation of dopamine release by NBQX. One possibility is that AMPA receptors located on cortical pyramidal neurons projecting to the midbrain and brainstem are inhibited by NBQX, thus causing less glutamatergic drive of AMPH-stimulated mesocortical, raphe-cortical and coeruleus-cortical release. This view is in line with other work demonstrating that AMPH stimulates phasic release (Daberkow *et al.* 2013); therefore, reducing cell excitability with glutamate receptor antagonists would inhibit release events at the terminal level (i.e. in M1). These data support the idea that AMPA receptor antagonism might be a reasonable target for diseases in which overactivation of thalamocortical projections results in pathological movement. In fact the use of AMPA receptor antagonists has previously been shown to improve symptoms of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys rendered dyskinetic by chronic L-DOPA treatment (Konitsiotis *et al.* 2000). Our results suggest that cortical AMPA receptors may be responsible for those effects.

## Conclusions

Taken together, VL glutamate input acting on NMDA and AMPA receptors positioned on different populations of M1 elements (i.e. GABergic interneurons, pre-synaptic dopamine neurons and post-synaptic glutamate neurons) modulate basal and evoked cortical monoamine release dependent on the degree of activation. AMPH-stimulated monoamine release in M1 and hyperlocomotor activity appears to depend on the integrity of this thalamocortical signalling. In addition, these results also suggest that AMPH stimulates release via a TTX-independent mechanism.

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