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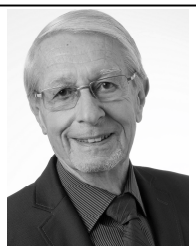
Use of Push-Pull Superfusion Technique for Identifying Neurotransmitters Involved in Brain Functions: Achievements and Perspectives

Michaela M. Kraus[1] and Athineos Philippu[2,*]

[1]*2nd Laboratory of Pharmacology, School of Medicine, Aristotle University of Thessaloniki, Greece;* [2]*Department of Pharmacology and Toxicology, University of Innsbruck, Austria*



A. Philippu

Abstract: The push-pull superfusion technique (PPST) is a procedure for *in vivo* examination of transmitter release in distinct brain areas. This technique allows to investigate dynamics of transmitter release both under normal and experimentally evoked conditions. The PPST can be modified so that it is possible to determine release of endogenous transmitters simultaneously with electroencephalogram (EEG) recordings, recordings of evoked potentials or the on-line determination of endogenous nitric oxide (NO) released into the synaptic cleft. Because of the good time resolution, the method provides further the possibility to modify the collection periods of superfusates depending on the neuronal function that is analyzed. For instance, investigation of central cardiovascular control, behavioral tasks or mnemonic processes requires very short collection periods, because changes in transmitter release occur within seconds. Even more important is the time resolution when rates of transmitter release are correlated with evoked extracellular potentials or EEG recordings. This review provides an overview of the different devices which might be combined with the PPST and perspectives for future work.

Keywords: Behaviour, central cardiovascular control, electroencephalogram (EEG), evoked potentials, neurotransmitters, nitric oxide (NO) on-line determination, push-pull superfusion technique (PPST), oscillations, ultradian rhythm.

INTRODUCTION

Determination of transmitter release in distinct brain areas is a prerequisite for understanding how brain is functioning, which changes in neuronal function are responsible for various central diseases and, therefore, it is an indispensable tool for the development of specific drugs for their treatments.

Several procedures exist for this purpose. Among them push-push superfusion technique (PPST) [1] and microdialysis [2] have the advantage that they provide information not only about overall changes in neuronal activity within distinct brain areas, but they additionally show quantitative alterations in transmitter release rates. Very important, with these techniques the dynamics of transmitter release under normal and experimentally evoked conditions is recorded. Both procedures and particularly PPST are more demanding and time consuming than most of the other procedures which are used. On the other hand, the more demanding and time consuming the procedures, the more exact and valuable the information they provide concerning involvement of transmitters in brain function and dysfunction.

The push-pull cannula (PPC) has been developed by Gaddum [3] and used for the determination of transmitter release in several tissues. The principle is as following: Locke or a similar solution is pushed through one of two parallel [3] or concentric [4] needles and pulled out from the second needle, the system being working like a siphon. When concentric needles or cannulae are used, the inner one protrudes to some extent. Substances locally released are determined in the perfusing fluid. This principle might be useful in peripheral tissues. However, because of the protruding inner needle in the brain and the malfunctioning siphon less fluid is pulled out of the brain than pushed in it. This leads to a local pressure increase in the perfused area that dramatically changes blood pressure, respiration, and other brain functions depending on the area that is perfused. In turn, these functional changes influence dramatically the release rates of transmitters and modulators thus rendering impossible the determination of transmitter release under normal physiological conditions. The development of a modified PPC and PPST was necessary so as to determine the dynamics of neurotransmitter release in the brain under normal physiological conditions, as well as experimentally induced conditions. The main characteristic of the modified PPC is that the inner needle ends about 10 mm above the outer needle so that the brain tissue is smoothly *superfused,* tissue damage is slight and brain functions remain unchanged [1].

In this review the modified PPCs and PPST will be described which have been successfully used for determination of neurotransmitters released in distinct brain areas, as well as the dynamics of transmitter release under 1. normal conditions, 2. during experimentally induced blood pressure changes, 3. during recording of the electro-encephalogram (EEG), 4. during recording of evoked

*Address correspondence to this author at the Department of Pharmacology and Toxicology, University of Innsbruck, Innrain 80-82, 6020 Innsbruck Austria; Tel: ++43 512 280489; Fax: ++43 512 280489;
E-mail: athineos.philippou@uibk.ac.at

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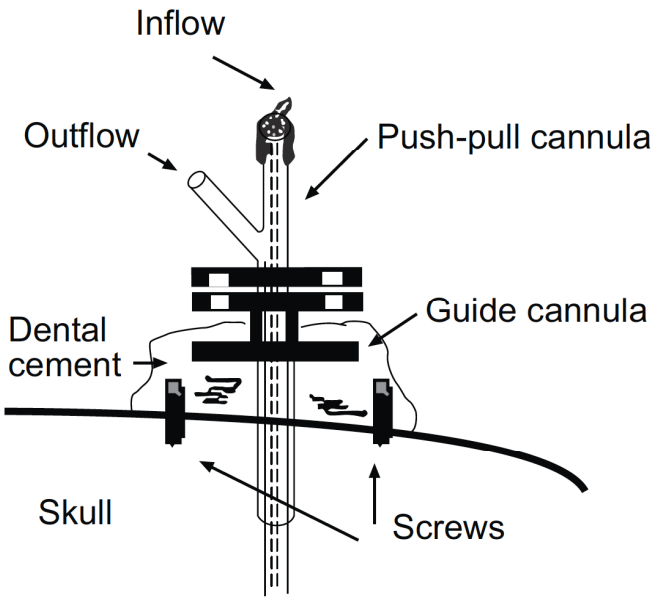

Fig. (1). The Push-pull cannula (PPC) and its guide cannula. Both cannulae are made of metal (guide cannula: o.d. 1.25 mm, i.d. 0.90 mm; PPC: outer cannula: o.d. 0.80 mm, i.d. 0.50 mm, inner cannula: o.d. 0.20 mm, i.d. 0.10 mm). The PPC is 2 mm longer than its guide cannula, thus reaching the investigated brain area. The outer cannula of the PPC protrudes about 1mm [5].

potentials, 5. during real-time determination of nitric oxide (NO) and 6. when investigations on behavior were performed. Experiments were carried out either in anaesthetized or conscious, freely moving animals.

SPONTANEOUS RELEASE OF NEURO-TRANSMITTERS: ULTRADIAN RHYTHMS

PPC [1,5] is shown in Fig. **1**.

In anaesthetized and conscious, freely moving animals, the release rates of several transmitters in various brain regions are not constant but oscillate according to ultradian rhythms. For example, in the nucleus of the solitary tract of the anaesthetized cat the release rates of catecholamines fluctuate according to a low frequency rhythm (1 cycle/1 hour) and a high frequent rhythm (1 cycle/10 min) (Fig. **2**) [6].

In the mamillary body and the medial amygdaloid nucleus of the cat the release rate of histamine also oscillates with a low frequency rhythm of 1 cycle/90 min, 1 cycle/135 min, respectively. Additionally, high frequency oscillations (1 cycle/19 min) exist in both areas [7].

Similar oscillations have been found in the hypothalamus of the conscious, freely moving rabbit [8]. These oscillations reflect changes in neuronal activity and are very probably due to fluctuations in presynaptic transmitter modulation.

Recently, it has been shown that in mice the circadian nuclear receptor REV-ERBα, that is associated with bipolar disorders, impacts midbrain dopamine production and mood-related behaviour [9]. It is intriguing to postulate that a similar receptor is also involved in the genesis of the ultradian oscillations described above.

CENTRAL CARDIOVASCULAR CONTROL: EFFECTS OF EXPERIMENTALLY INDUCED BLOOD PRESSURE CHANGES

Neuronal biogenic amines as catecholamines and serotonin located in various brain areas are responsible for arterial blood homeostasis [10-14]. The most direct way to identify brain neurons involved in central blood pressure control is to investigate, whether experimentally induced blood pressure changes lead to counteracting changes in neuronal activities [10, 12].

In anaesthetized cats, superfusion of the posterior hypothalamus and collection of superfusates in very short periods of 10 s reveals that temporal differences exist in the counteracting release of catecholamines, when blood pressure is experimentally lowered. Intravenous injection of nitroprusside leads to a fall of blood pressure which is combined with an immediate increase in the release of dopamine. The release of adrenaline is enhanced 60 s later than that of dopamine, while the release of noradrenaline is increased after a delay of about 70 s (Fig. **3**).

Similar results have been obtained, when hypotension was elicited by controlled bleeding [15].

These findings underline the necessity of an optimal time resolution when the role of central neurons containing various neurotransmitters in brain functions is investigated. In comparison with other techniques used to study the *in vivo* release of transmitters, the modified PPC has the best time resolution because the brain tissue is directly superfused with the superfusing fluid without interference of a semi-permeable membrane [16].

Besides biogenic amines, inhibitory and excitatory amino acids are involved in blood pressure regulation [10]. In the locus coeruleus of conscious, freely moving rats a fall of blood pressure provoked by i.v. injection of nitroprusside enhances the release rate of the excitatory amino acid glutamate, so as to counteract the hypotension, without influencing that of GABA and arginine (Fig. **4**).

On the other hand, increase in blood pressure elicited by i.v. injection of noradrenaline elicits a counteracting increase in the release of the endogenous inhibitory amino acid GABA without influencing the release rates of the excitatory amino acid glutamate or arginine (Fig. **5**).

It is interesting to mention that in sino-aortic denervated (SAD) rats, the pressor response to noradrenaline does not influence the release of GABA in the locus coeruleus showing that the GABAergic response is mediated *via* peripheral baroreceptors. Moreover, in SAD animals the basal release rate of glutamate greatly augments blood pressure lability [11].

SIMULTANEOUS DETERMINATION OF TRANS-MITTER RELEASE AND ELECTROENCEPHALOGRAM (EEG) RECORDINGS IN A DISTINCT BRAIN AREA

The release of histamine in the posterior hypothalamus (PH) is determined simultaneously with the extracellular

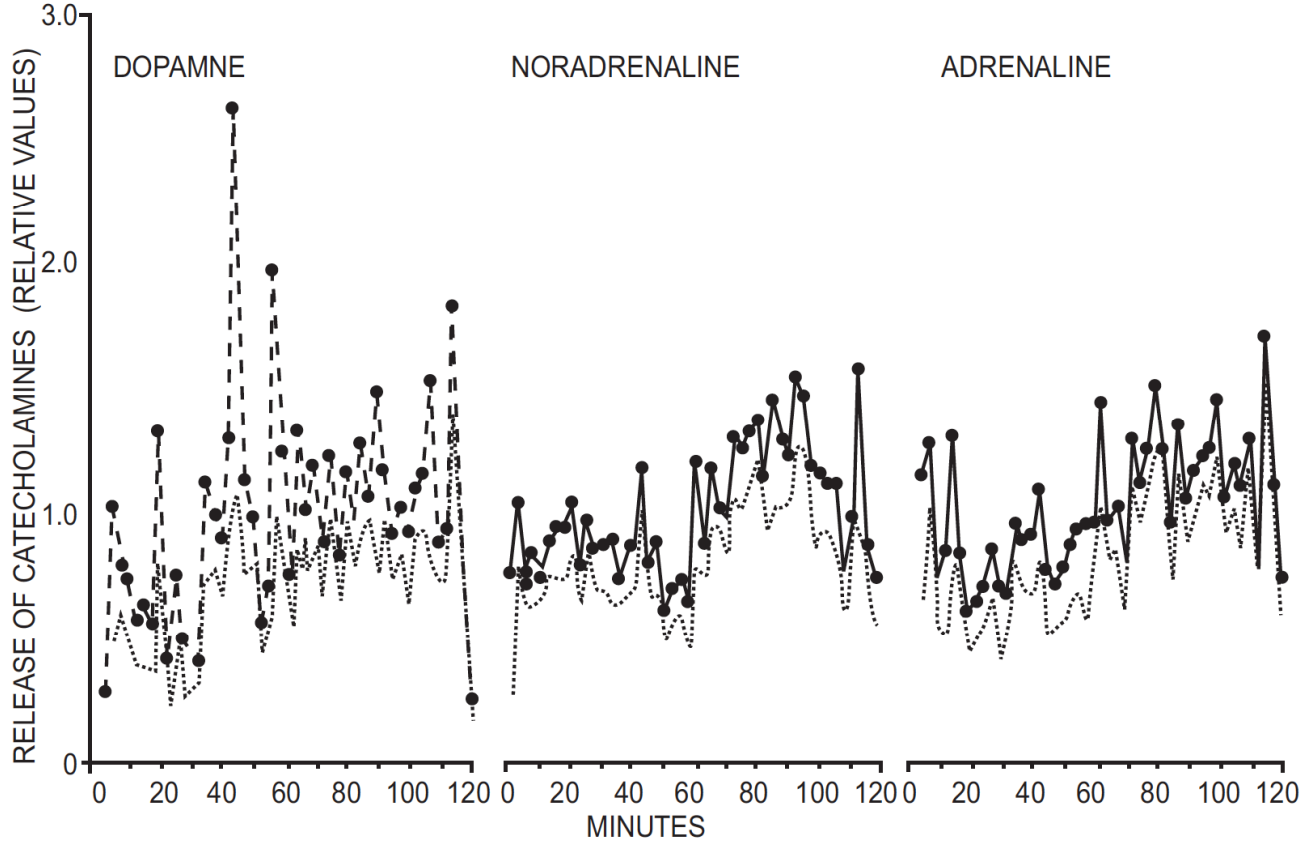

Fig. (2). Release rates of catecholamines in the nucleus of the solitary tract of the anaesthetized cat. The superfusates are collected in time periods of 2.5 min. The mean release of each experiment is taken as 1.00. Open circles denote fixed last peaks of high release rates. Mean values ± S.E.M. as dotted line. Dopamine n=7, noradrenaline n=10, adrenaline n=9 [6].

EEG recording. For this purpose, a microelectrode (0.5 MΩ impedance, tungsten monopolar insulated microelectrode, shaft diameter 0.216 mm, tip diameter 1-2 µm) [17] is inserted into the outer tubing of the non-metallic PPC. A negative correlation exists between histamine release and the ultradian rhythm in the delta and theta frequency bands of the EEG. Indeed, low release rate of histamine corresponds to states of high neuronal activity within the PH [17]. H_3 receptor antagonists applied intracerebroventricularily (i.c.v.) inhibit experimentally evoked seizures evoked by electrodes implanted into the amygdala [18]. Besides histaminergic, catecholaminergic and dopaminergic neurons influence the ultradian EEG rhythm in the rat PH [19, 20].

SIMULTANEOUS DETERMINATION OF TRANSMITTERS AND EVOKED POTENTIALS IN A DISTINCT BRAIN AREA

For *in vivo* electrochemical characterization of the projection from distinct brain areas to other brain structures paired pulses are electrically evoked and incoming signals are recorded as extracellular potentials. Using the PPC the biochemical transmission is investigated simultaneously. A parylene-coated tungsten-electrode (2.0 MΩ, outside diameter: 0.22mm) is placed inside the outer needle of the PPC. Additionally, a concentric bipolar stimulation electrode (0.25mm exposed) is inserted to the brain structure to be stimulated [21].

Superfusion of the dorsolateral NAc of anaesthetized rats with the nitric oxide (NO) synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 500 µM) attenuates potentials evoked by electrical stimulation of the lateral aspect of the parafascicular thalamus (Pf) and decreases glutamate, aspartate, and GABA release, while the NO donor 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine (PAPA/NO, 500 µM) exerts the opposite effect [21]. NOS containing neurons seem not to be in synaptic contact with thalamic fibers [22] but fibers arising from the Pf are directly in synaptic contact with striatal cholinergic interneurons [23, 24] which are known to activate NOS positive cells [25].

Thus, PPST together with simultaneous recording of evoked potentials makes it possible to investigate neurotransmitter release during activation of different afferences to a small brain area. In this way, projections to distinct areas of the brain and their functional significance might be elucidated. Moreover, the influence of neuroactive substances applied locally is accurately investigated.

ON-LINE RECORDING OF NITRIC OXIDE IN A DISTINCT BRAIN AREA

An amperometric microelectrode introduced into the outer tube of the PPC allows real-time monitoring of NO simultaneously with determination of neurotransmitters and local application of neuroactive substances [26]. In anaesthetized rats, superfusion of the NAc with tetrodotoxin

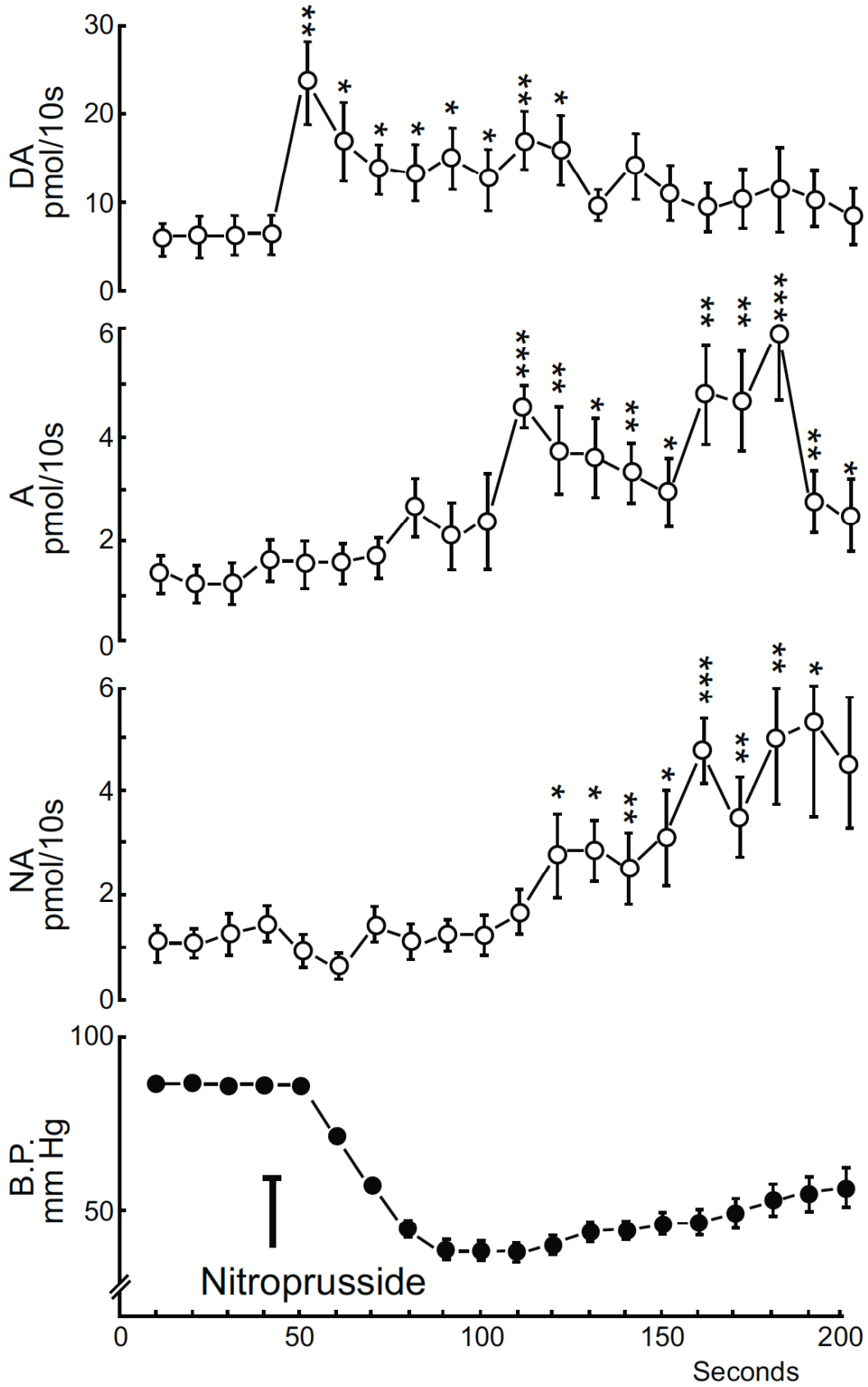

Fig. (3). Effects of sodium nitroprusside (5 µg kg^{-1}, i.v.) on release rates of catecholamines in the posterior hypothalamus and mean arterial blood pressure in anaesthetized cats. The samples are collected every 10 s. Noradrenaline (NA), dopamine (DA), adrenaline (A). Mean values of five experiments ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001 [15].

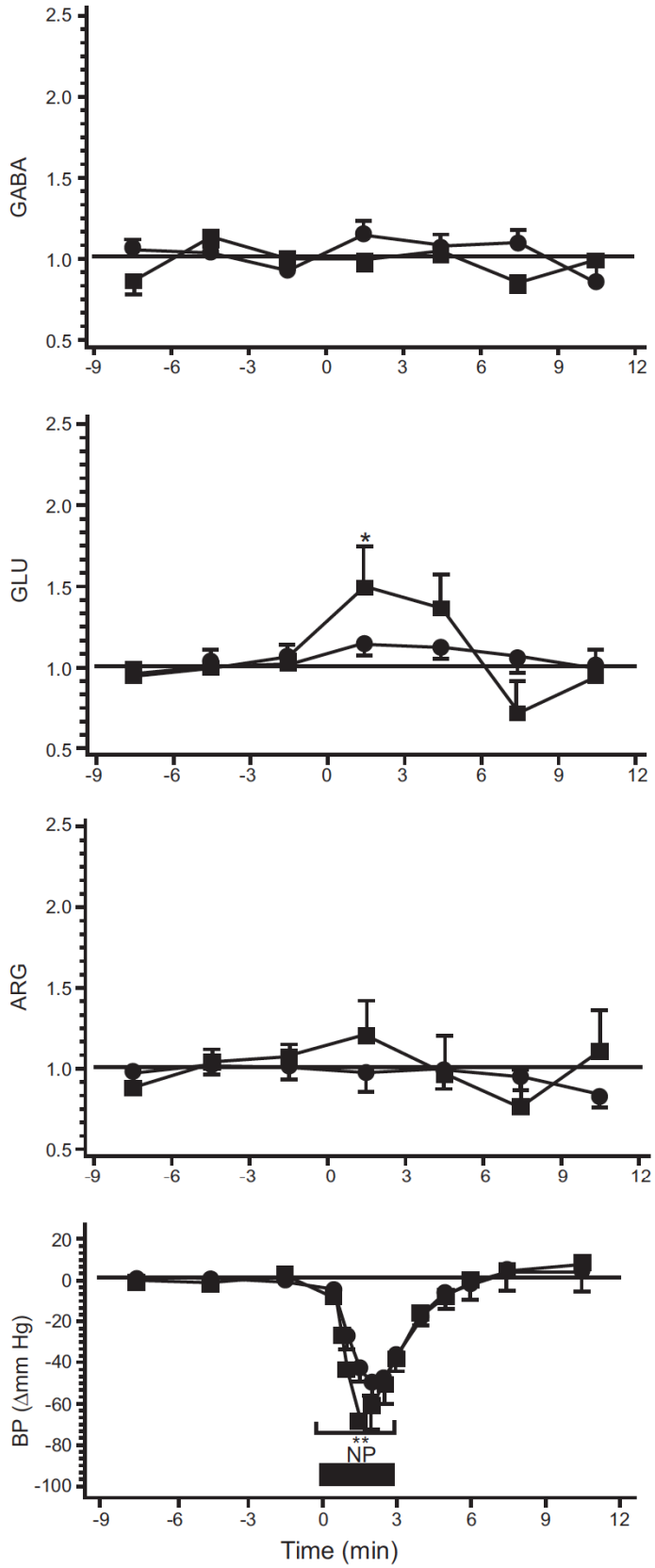

Fig. (4). Effects of sodium nitroprusside (NP; 150 μg kg^{-1} min^{-1}, i.v.) on arterial blood pressure (BP), GABA, glutamate (GLU) and arginine (ARG) release in the locus coeruleus of conscious, freely moving rats. The mean release rates in three samples preceding infusion of NP are taken as 1.0. Horizontal bar denotes the onset and duration of NP infusion. Circles: SAD rats (n=5), squares: sham-operated rats (n=9). Mean values ± S.E.M. *$p < 0.05$ [11].

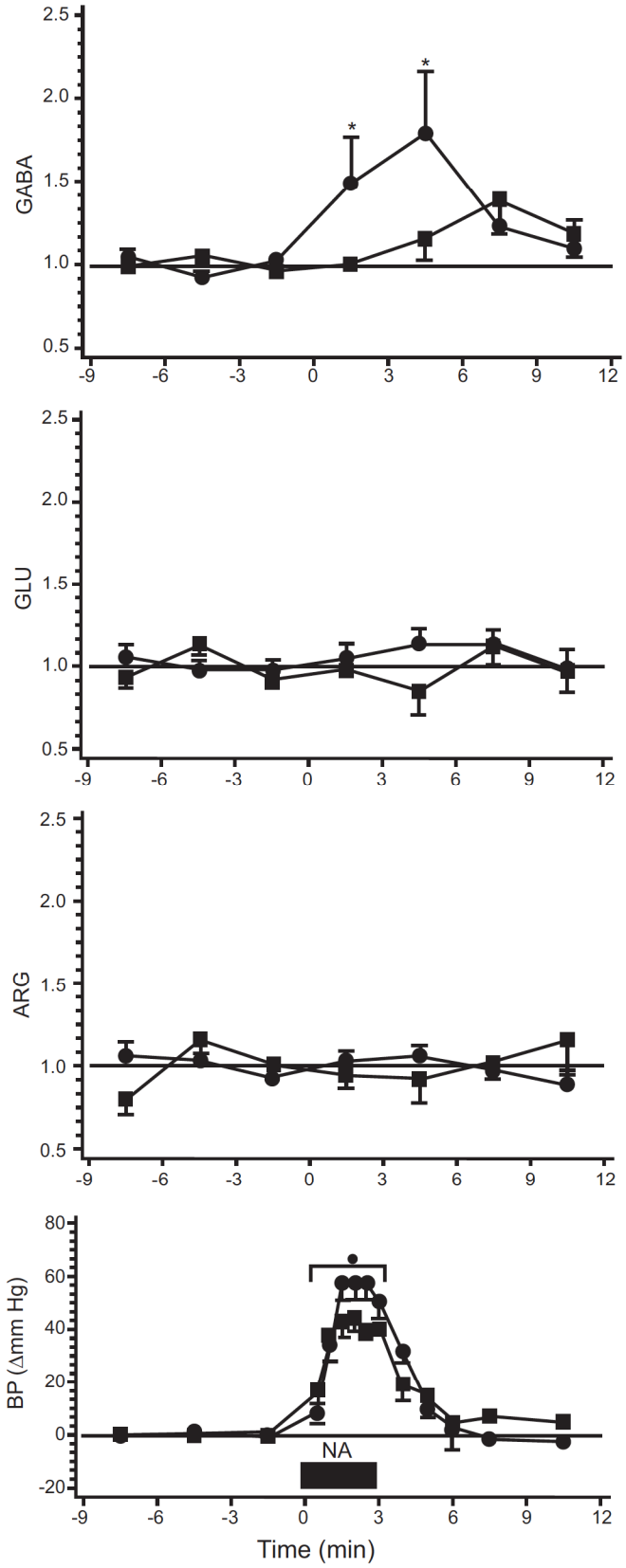

Fig. (5). Effects of noradrenaline (NA; 4 µg kg^{-1} min^{-1}) on arterial blood pressure (BP), GABA, glutamate (GLU) and arginine (ARG) release in the locus coeruleus of conscious, freely moving rats. The mean release rates in the three samples prior infusion with NA are taken as 1.0. Horizontal bar shows onset and duration of infusion with NA. Squares: SAD rats (n=9), circles: sham-operated rats (GABA: n=9; glutamate: n=10; arginine: n=9). Mean values ± S.E.M. *$p < 0.05$ [11].

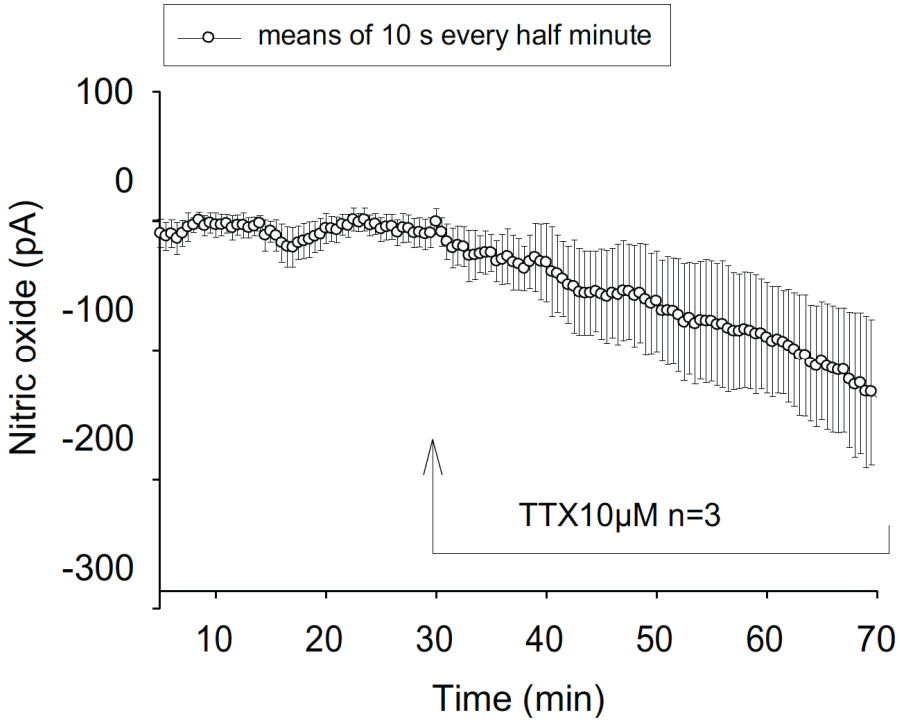

Fig. (6). Effect of superfusion of the NAc of anaesthetized rats with TTX (10 µM) on NO release. NO-related electrochemical potential is shown as means (circles) ± SEM (bars) calculated every 30 seconds. The potential of 0 mV relates to the basal NO release. [26].

(TTX, 10 µM) *via* the PPC decreases NO levels in the superfusate (Fig. **6**) [26].

To stimulate NO synthesis, the NAc is superfused with NMDA (50 µM). The enhanced NO synthesis leads in turn to an increased NO release [26].

These findings confirm the accuracy of NO signal recordings and the feasibility of simultaneous online determination of NO together with release of neuro-transmitters. The labile NO electrode is thereby protected by the PPC during inserting the devices into the investigated brain area. The superfusate is free of blood, hemoglobin and cell debris which might influence NO concentration. Furthermore, physiological conditions are maintained for several hours.

ASSESSMENT OF NEUROTRANSMITTER RELEASE DURING MNEMONIC PROCESSES

The olfactory, social memory test [27] is based on the chemosensorily mediated memory of an animal for a social conspecific [28]. It is based on the time needed by an adult, sexually experienced rat to become acquainted with an unfamiliar juvenile rat, the time required being an index of short-term memory. When re-exposure is carried out in less than one hour, the adult rat recognizes the juvenile rat and contact time is short [27]. An interval of two hours between the two exposures requires an entire investigation of the juvenile by the adult rat so that contact time to recognition during first and second exposure are similar [29]. Combination of transmitter release determination by the PPC with the olfactory, social memory test makes it possible to correlate neuronal activity in a distinct brain area with memory processes.

Learning processes initiated during performance of the olfactory, social memory test are associated with enhanced release rates of glutamate and ACh in the NAc. On the other hand, recognition that reflects short-term memory is ineffective (Fig. **7**) [30].

These findings are consistent with the observation that damage to the NAc only slightly affects retention of acquired place navigation in the Morris water task [31]. Moreover, acquisition of passive-avoidance learning rather than maintenance of memory is affected when cholinergic transmission is attenuated [32, 33].

The significance of histaminergic projections within cholinergic and other neuronal circuits on mnemonic processing is well established [34-44] and has also been investigated by PPST [34, 37, 39, 40]. During learning processes i.c.v. injection of the H_3 receptor antagonist thioperamide or the H_2 receptor antagonist famotidine greatly shortens recognition time while the release rates of ACh and glutamate are not changed. The findings support the idea that within the NAc histaminergic neurons facilitate short-term memory without affecting release of ACh and glutamate [30].

Furthermore, PPST might be used for investigating transmitter release in models of anxiety and pain [45] since the fixation of the cannula onto the skull is tight enough to resist to intense movements of the animal during different behavioral tasks.

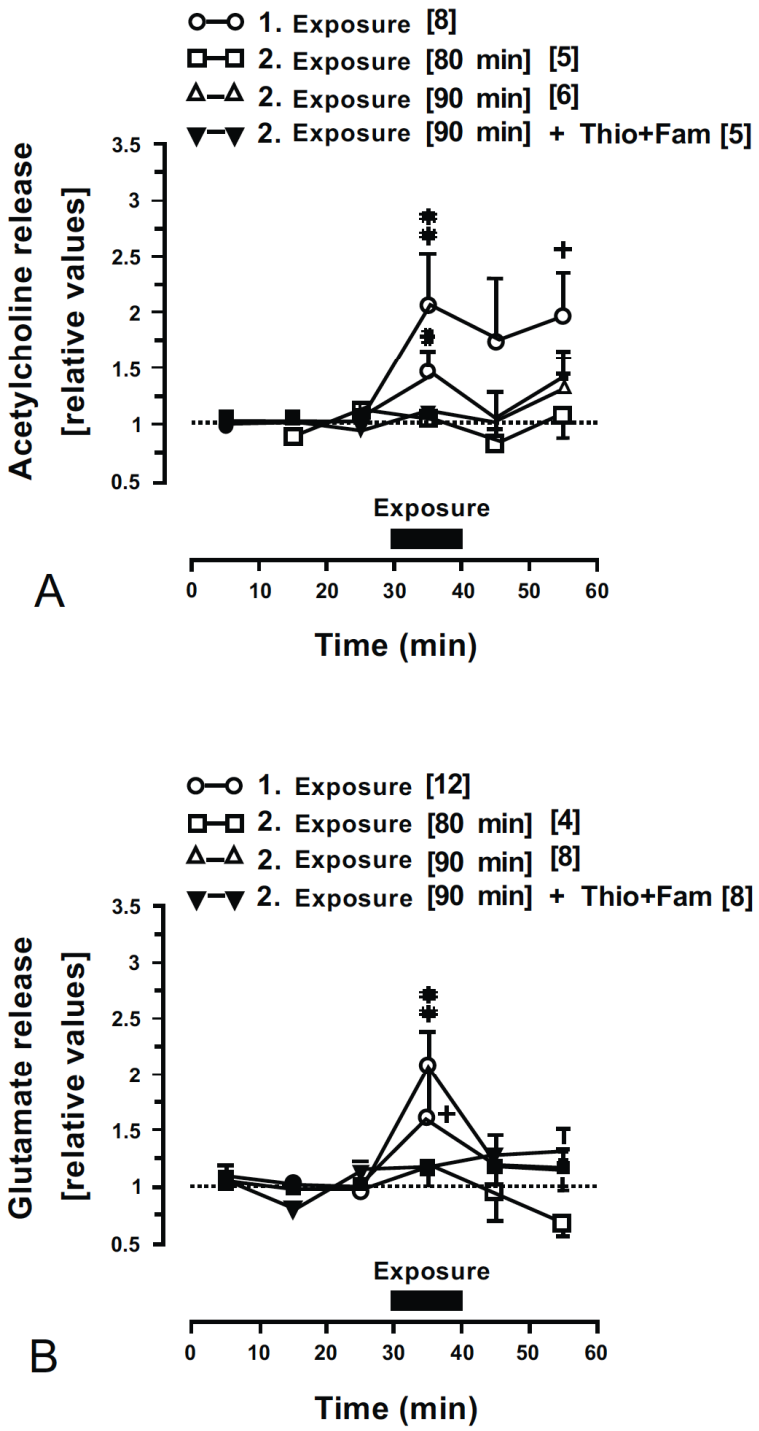

Fig. (7). Release of ACh (A) and glutamate (B) in the NAc during performance of the olfactory, social memory task in rats. Effect of i.c.v. injection of 5 µg thioperamide (Thio) together with 20 µg famotidine (Fam) on transmitter release. Histamine receptor antagonists or aCSF (vehicle) is injected immediately after the first exposure. Each exposure lasts 10 min and second exposure takes place 60 or 90 min after the first exposure. Horizontal bars indicate exposure of the juvenile rat to the adult rat. Basal release rate in the three samples preceding the exposure is taken as 1. Mean values ± SEM. Number of rats is indicated in parentheses. *$p < 0.05$; **$p < 0.01$, significantly different from basal release rate [30].

PRESYNAPTIC TRANSMITTER MODULATION

Findings obtained during superfusion of the NAc with various receptor agonists and antagonists are summarized in Fig. **8**.

A transmitter, for example histamine, is released from its neuron in the synaptic cleft and *via* auto- and heteroreceptors either enhances or inhibits the activity of its own neuron and of neighbouring neurons. This micro system is influenced by

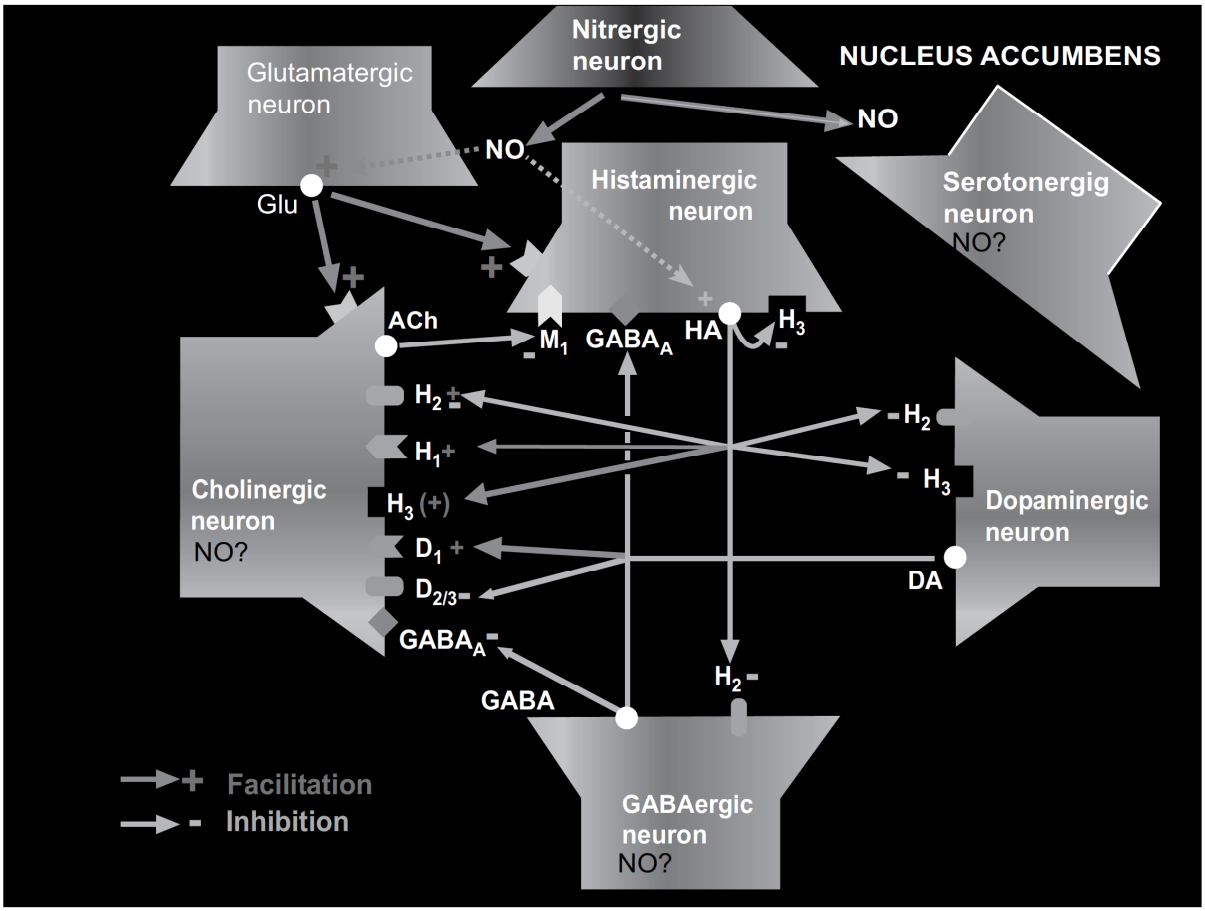

Fig. (8). Presynaptic modulatory processes in the synaptic cleft of the NAc. Modified from [46].

NO which is liberated from nitrergic neurons and either directly or indirectly *via* glutamatergic neurons modulates the transmitter release in the synapse [46-48].

EVALUATION AND PERSPECTIVES

Combination of PPC with various types of electrodes makes it possible to record locally EEG or extracellular potentials simultaneously with the determination of transmitter release. The great advantage of this procedure is that electrodes are placed directly in the area in which transmitters are released. This is of crucial importance because of the small areas that are studied. Insertion of the electrode parallel to the area in which the transmitter release is assessed probably leads to potential recordings outside of this area in which the transmitter release is investigated. Placement of the electrode within the superfused area and good resolution time, which is indispensable for correlation of neuronal activity with brain function, are the main advantages of the PPST when compared to microdialysis. On the other hand, the main disadvantage of PPST is that cannulae obtained commercially should be carefully tested before use because they may not work appropriately. This renders PPST more demanding that microdialysis.

For commercial purposes a new PPC is under construction. It consists of high technology polymer and possesses an integrated tissue barrier.

The advantage of the PPST combined with the NO electrode is the continuous determination of the NO concentration in the tissue with a time resolution of less than 5 seconds.

The described techniques and assays make it possible to investigate the involvement of transmitter release in various brain functions from central blood pressure control to behavior and presynaptic modulation of transmitter release. The knowledge of these mutual interchanges under physiological conditions and in brain disorders is a prerequisite for the development of selective drugs for their therapy.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

[1] Philippu, A. Use of push-pull cannulae to determine the release of endogenous neurotransmitters in distinct brain areas of anaesthetized and freely moving animals. In: *Measurement of Neurotransmitter release;* Marsden, C.A., Ed.; John Wiley: Chichester, **1984**, 3-37.

[2] Chefer, V.I.; Thompson, A.C.; Zapata, A.; Shippenberg, T.S. Overview of brain microdialysis. *Curr. Protoc. Neurosci.*, **2009**, *7*, Unit 7.1. Doi: 10.1002/0471142301. ns 0701s47. http://dx.doi.org/10.1002/0471142301

[3] Gaddum, J.H. Push-pull cannulae. *J. Physiol.* (London), **1961**, *155* (suppl), 1P-2P.

[4] Szerb, J.C. Model experiments with Gaddum's push-pull cannulas. *Can. J. Physiol. Pharmacol.,* **1967**, *45(4)*, 613-620. http://dx.doi.org/10.1139/y67-074

[5] Kraus, M.M. Study of nitric oxide- and histamine- mediated *in vivo* release of neurotransmitters in the ventral striatum; role of the ventral striatum in behaviour, memory and neurotoxicity of amphetamine. PhD Thesis, University of Innsbruck: Innsbruck, June **2001**.

[6] Lanzinger, I.; Kobilansky, C.; Philippu, A. Pattern of catecholamine release in the nucleus tractus solitarii of the cat. *Naunyn-Schmiedeberg's Arch. Pharmacol*., **1989**, *339(3)*, 298-301. http://dx.doi.org/10.1007/BF00173581

[7] Prast, H.; Saxer, A.; Philippu, A. Pattern of *in vivo* release of endogenous histamine in the mamillary body and the amygdala. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1988**, *337(1)*, 53-57. http://dx.doi.org/10.1007/BF00169476

[8] Philippu, A.; Hanesch, U.; Hagen, R.; Robinson, R.L. Release of endogenous histamine in the hypothalamus of anaesthetized cats and conscious, freely moving rabbits. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1982**, *321(4)*, 282-286. http://dx.doi.org/10.1007/BF00498514

[9] Chung, S.; Lee, E.J.; Yun, S.; Choe, H.K.; Park, S.-B.; Son, H.J.; Kim, K.-S.; Dluzen, D.E.; Lee, I.; Hwang, O.; Son, G.H.; Kim, K. Impact of circadian nuclear receptor REV-ERBα on midbrain dopamine production and mood regulation. *Cell*. **2014**, *157(4)*, 858-868. doi: 10.1016/j. cell.2014.03.039.

[10] Singewald, N.; Philippu, A. Involvement of biogenic amines and amino acids in central regulation of cardiovascular homeostasis. *Trends Pharmacol. Sci.*, **1996**, *17(10),* 356-363. http://dx.doi.org/10.1016/S0165-6147(96)80009-5

[11] Kouvelas, D.; Singewald, N.; Kaehler, S.T.; Philippu, A. Sinoartic denervation abolishes blood pressure-induced GABA release in the locus coeruleus of conscious rats. *Neurosci. Lett.*, **2006**, *393(2-3),* 194-199. http://dx.doi.org/10.1016/j.neulet.2005.09.063

[12] Philippu, A. Involvement of hypothalamic catecholamines in the regulation of the arterial blood pressure. *Trends Pharmacol. Sci.*, **1980**, *1,* 376-378. http://dx.doi.org/10.1016/0165-6147(80)90054-1

[13] Dampney, R.A. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev.*, **1994**, *74(2)*, 323-364.

[14] Guyenet, P.G. The sympathetic control of blood pressure. *Nat. Rev. Neurosci.,* **2006**, *7(5)*, 335-346. http://dx.doi.org/10.1038/nrn1902

[15] Sinha, J.N.; Dietl, H.; Philippu, A. Effect of a fall of blood pressure on the release of catecholamines in the hypothalamus. *Life Sci.*, **1980**, *26(21),* 1751-1760. http://dx.doi.org/10.1016/0024-3205(80)90574-3

[16] Philippu, A. Ed. Synaptic events in brain function. Approaches to synaptic events *in vivo* under physiological and pathological conditions: State of the art, Wiley-Blackwell Copenhagen, Denmark, 17-23 July 2010; Proceedings of IUPHAR; W 33.1, 135, **2010**.

[17] Prast, H.; Grass, K.; Philippu, A. The ultradian EEG rhythm coincides temporally with the ultradian rhythm of histamine release in the posterior hypothalamus. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1997**, *356(4)*, 526-528. http://dx.doi.org/10.1007/PL00005086

[18] Harada, C.; Hirai, T.; Fujii, Y.; Harusawa, S.; Kurihara, T.; Kamei, C. Intracerebroventricular administration of histamine H3 receptor antagonists decreases seizures in rat models of epilepsia. *Methods Find. Exp. Clin. Pharmacol.*, **2004**, *26*(4), 263-270.

[19] Prast, H.; Grass, K.; Philippu, A. Influence of histamine receptor agonists and antagonists on ultradian rhythm of EEG in the posterior hypothalamus of the rat. *Neurosci. Lett.*, **1996**, *216*(1), 21-24. http://dx.doi.org/10.1016/0304-3940(96)12992-X

[20] Grass, K.; Prast, H.; Philippu, A. Influence of catecholamine receptor agonists and antagonists on the ultradian rhythm of the EEG in the posterior hypothalamus. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1998**, *357*(2), 169-175. http://dx.doi.org/10.1007/PL00005151

[21] Kraus, M.M.; Prast, H.; Philippu, A. Influence of parafascicular thalamic input on neuronal activity within the nucleus accumbens is mediated by nitric oxide - an *in vivo* study. *Life Sci.*, **2014**, *102(1)*,49-54. doi: 10.1016/j.lfs.2014.02.029. http://dx.doi.org/10.1016/j.lfs.2014.02.029

[22] Kachidian, P.; Vuillet, J.; Nieoullon, A.; Laffaille, G.; Kerkerian-Le Goff, L. (1996) Striatal neuropeptide Y neurons are not a target for thalamic afferent fibres. *Neuroreport*, **1996**, *7(13)*, 1665-1669. http://dx.doi.org/10.1097/00001756-199607080-00028

[23] Lapper, S.R.; Bolam, J.P. Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. *Neuroscience*, **1992**, *51(3)*, 533-545. http://dx.doi.org/10.1016/0306-4522(92)90293-B

[24] Meredith, G.E.; Wouterlood, F.G. Hippocampal and midline thalamic fibers and terminals in relation to the choline acetyltransferase-immunoreactive neurons in nucleus accumbens of the rat: a light and electron microscopic study. *J. Comp. Neurol.*, **1990**, *296(2),* 204-221. http://dx.doi.org/10.1002/cne.902960203

[25] Vuillet, J.; Dimova, R.; Nieoullon, A.; Kerkerian-Le Goff, L. Ultrastructural relationships between choline acetyltransferase- and neuropeptide Y-containing neurons in the rat striatum. *Neuroscience*, **1992**, *46(2)*, 351-360. http://dx.doi.org/10.1016/0306-4522(92)90057-9

[26] Prast, H.; Hornick, A.; Kraus, M.M.; Philippu, A. Origin of endogenous nitric oxide released in the nucleus accumbens under real-time *in vivo* conditions. *Life Sci,* (in press). http://dx.doi.org/10.1016/j.lfs.2015.04.021

[27] Thor, D.H.; Holloway, W.R. Social memory of the male laboratory rat. *J. Comp. Physiol. Psychol.,* **1982**, *96*, 1000-1006. http://dx.doi.org/10.1037/0735-7036.96.6.1000

[28] Setlow, B. The nucleus accumbens and learning and memory. *J. Neurosci. Res.,* **1997**, *49*, 515-521. http://dx.doi.org/10.1002/(SICI)1097-4547(19970901)49:5<515::AID-JNR1>3.0.CO;2-E

[29] Dantzer, R.; Bluthe, R.M.; Koob, G.F.; Le Moal, M. Modulation of social memory in male rats by neurohypophyseal peptides. *Psychopharmacology Berl.,* **1987**, *91*, 363-368. http://dx.doi.org/10.1007/BF00518192

[30] Kraus, M.M.; Prast, H.; Philippu, A. Facilitation of short-term memory by histaminergic neurons in the nucleus accumbens is independent of cholinergic and glutamatergic transmission. *Br. J. Pharmacol.*, **2013**, *170(1)*, 214-221, doi: 10.1111/bph.12271. http://dx.doi.org/10.1111/bph.12271

[31] Sutherland, R.J.; Rodriguez, A.J. The role of the fornix/fimbria and some related subcortical structures in place learning and memory. *Behav. Brain Res.*, **1989**, *32(3)*, 265-277. http://dx.doi.org/10.1016/S0166-4328(89)80059-2

[32] Ikarashi, Y.; Kuribara, H.; Shiobara, T.; Takahashi, A.; Ishimaru, H.; Maruyama, Y. Learning and memory in mice treated with choline oxidase, a hydrolytic enzyme for choline. *Pharmacol. Biochem. Behav.*, **2000**, *65(3)*, 519-522. http://dx.doi.org/10.1016/S0091-3057(99)00223-3

[33] Robinson, L.; Platt, B.; Riedel, G. Involvement of the cholinergic system in conditioning and perceptual memory. *Behav. Brain Res.*, **2011**, *221(2)*, 443-465. doi: 10.1016/j.bbr.2011.01.055. http://dx.doi.org/10.1016/j.bbr.2011.01.055

[34] Philippu, A.; Prast, H. Importance of histamine in modulatory processes, locomotion and memory. *Behav. Brain Res.*, **2001**, *124(2)*, 151-159. http://dx.doi.org/10.1016/S0166-4328(01)00226-1

[35] Alvarez, E.O. The role of histamine on cognition. *Behav. Brain Res.*, **2009**, *199(2)*, 183-189. doi: 10.1016/j.bbr.2008.12.010. http://dx.doi.org/10.1016/j.bbr.2008.12.010

[36] Zlomuzica, A.; Ruocco, L.A.; Sadile, A.G.; Huston, J.P.; Dere, E. Histamine H1 receptor knockout mice exhibit impaired spatial memory in the eight-arm radial maze. *Br. J. Pharmacol.*, **2009**, *157(1)*, 86-91. doi: 10.111/j.1476-5381.2009.00225.x.

[37] Prast, H.; Argyriou, A.; Philippu, A. Histaminergic neurons facilitate social memory in rats. *Brain Res.*, **1996**, *734(1-2)*, 316-318.http://dx.doi.org/10.1016/0006-8993(96)00886-4

[38] Alleva, L.; Tirelli, E.; Brabant, C. Therapeutic potential of histaminergic compounds in the treatment of addiction and drug-related cognitive disorders. *Behav Brain Res.*, **2013**, *237*, 357-368. doi: 10.1016/j.bbr.2012.09.025. http://dx.doi.org/10.1016/j.bbr.2012.09.025

[39] Prast, H.; Tran, M.H.; Fischer, H.; Kraus, M.; Lamberti, C.; Grass, K.; Philippu, A. Histaminergic neurons modulate acetylcholine release in the ventral striatum: role of H_3 histamine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1999**, *360(5)*, 558-564. http://dx.doi.org/10.1007/s002109900097

[40] Prast, H.; Tran, M.H.; Lamberti, C.; Fischer, H.; Kraus, M.; Grass, K.; Philippu, A. Histaminergic neurons modulate acetylcholine release in the ventral striatum: role of H_1 and H_2 histamine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1999**, *360(5)*, 552-557. http://dx.doi.org/10.1007/s002109900098

[41] Miyazaki, S.; Imaizumi, M.; Onodera, K. Effects of thioperamide, a histamine H3-receptor antagonist, on a scopolamine-induced learning deficit using an elevated plus-maze test in mice. *Life Sci.*, **1995**, *57(23)*, 2137-2144. http://dx.doi.org/10.1016/0024-3205(95)02206-X

[42] Onodera, K.; Miyazaki, S.; Imaizumi, M. Cognitive involvement by negative modulation of histamine H2 receptors in passive avoidance task in mice. *Methods Find. Exp. Clin. Pharmacol.*, **1998**, *20(4)*, 307-310. http://dx.doi.org/10.1358/mf.1998.20.4.485684

[43] Terry, A.V. Jr.; Buccafusco, J.J.; Herman, E.J.; Callahan, P.M.; Beck, W.D.; Warner, S.; Vandenhuerk, L.; Bouchard, K.; Schwarz, G.M.; Gao, J.; Chapman, J.M. The prototypical ranitidine analog JWS-USC-75-IX improves information processing and cognitive function in animal models. *J. Pharmacol. Exp. Ther.*, **2011**, *336(3)*, 751-766. http://dx.doi.org/10.1124/jpet.110.175422

[44] Köhler, C.A.; da Silva, W.C.; Benetti, F.; Bonini, J.S. Histaminergic mechanisms for modulation of memory systems. *Neural Plast.*, **2011**, 328602. doi: 10.1155/2011/328602. http://dx.doi.org/10.1155/2011/328602

[45] Singewald, N.; Kouvelas, D.; Kaehler, S.T.; Sinner, C.; Philippu, A. Peripheral chemoreceptor activation enhances 5-hydroxytryptamine release in the locus coeruleus of conscious rats. *Neurosci. Lett.*, **2000**, *289(1)*, 17-20. http://dx.doi.org/10.1016/S0304-3940(00)01241-6

[46] Philippu, A. Interactions of histamine with other neuron systems. In: *Histaminergic neurons. Morphology and function*; Wada, H.; Watanabe, T., Eds.; CRC Press: Boca Raton, **1991**, 323-343.

[47] Philippu, A.; Prast H. Role of histaminergic and cholinergic transmission in cognitive processes. *Drug News Perspect.*, **2001**, *14(9)*, 523-529. http://dx.doi.org/10.1358/dnp.2001.14.9.858409

[48] Philippu, A. Modulation by heteroreceptors of histamine release in the brain. In: *Annals of Psychiatry/Anuario Psiquiátrico; Basic and Clinical Neuroscience*; Cacabelos, R., Ed.; Prous Science Publishers: Barcelona, **1992**, *3*, pp. 79-87.

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