Verhaltensrelevanz neurochemischer Interaktionen zwischen dem pedunculopontinen tegmentalen Nucleus und den ventralen Basalganglien

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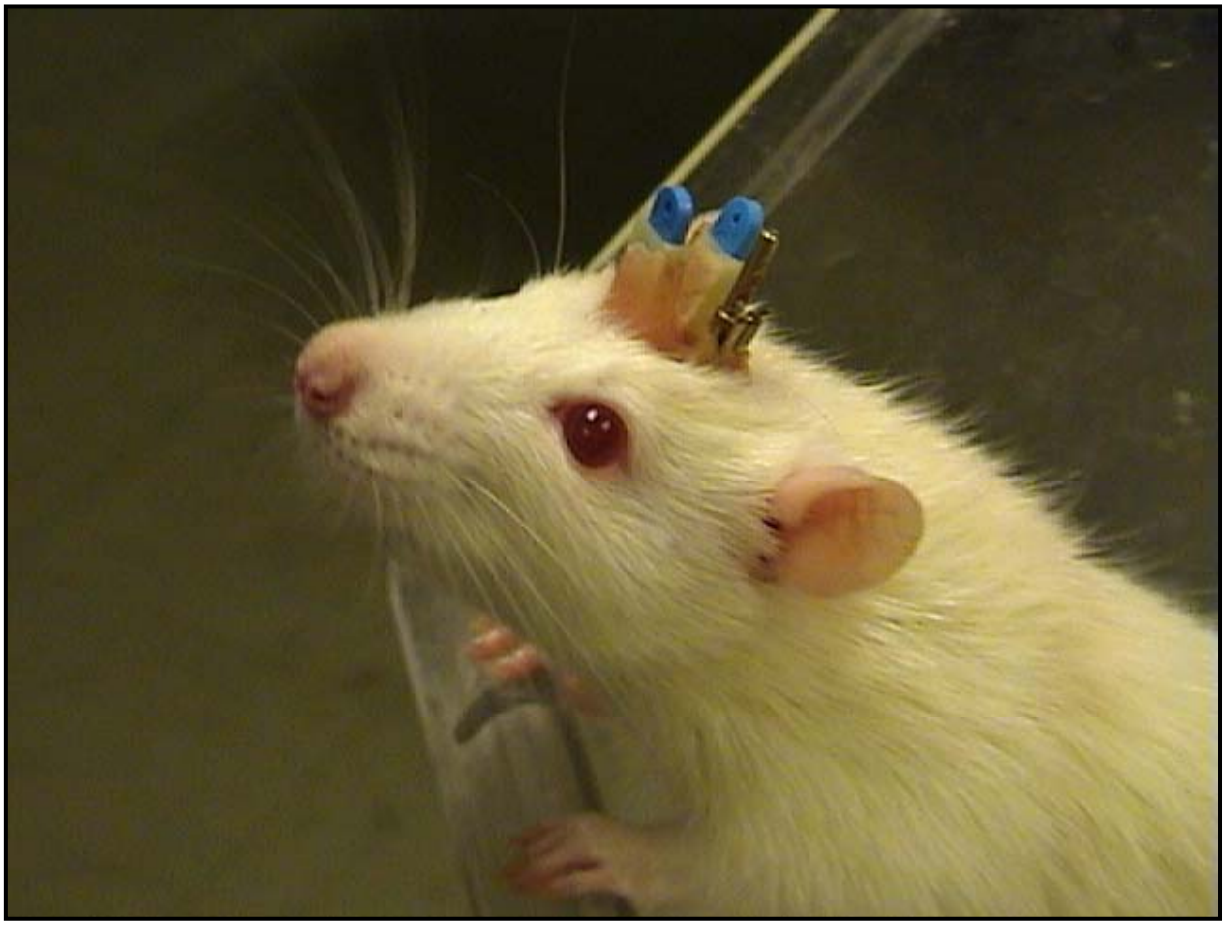

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1. Einleitung

1.1. Neurobiologische Grundlagen motorischer Verhaltensanpassungen

Tiere und Menschen passen ihr motorisches Verhalten sowohl den bestehenden als auch den sich ändernden Bedingungen ihrer Umwelt an. Um die Folgen einer Verhaltensantwort abzuwägen und entsprechend zu planen, wird interne motivationale und emotionale Information mit externer sensorischer Information verarbeitet. In diesem Prozess werden geeignete Verhaltensantworten verstärkt, während ungeeignete Verhaltensantworten gehemmt werden (*response selection*): Sieht z.B. ein mit der Nahrungsaufnahme beschäftigtes Beutetier ein Raubtier, dann induziert die sensorische Information (Raubtier) zusammen mit der internen Information (Gefahr, Furcht) die Hemmung des bestehenden Verhaltens (Nahrungsaufnahme) und eine geeignete neue Verhaltensantwort (Flucht) wird ausgelöst. Dieser Vorgang wird Verhaltensanpassung genannt. Bei Säugetieren werden das Verhalten und damit auch die Verhaltensanpassung über hierarchisch angeordnete motorische Bahnen im Gehirn gesteuert. An der Spitze dieser Bahnen steht der Kortex, der verhaltensrelevante Information sowohl direkt als auch indirekt über motorische Areale im Hirnstamm an die Motoneurone im Rückenmark leitet. Diese Motoneurone innervieren wiederum direkt die Skelettmuskulatur, die dann die motorische Verhaltensantwort ausführt. Zwei Systeme wirken modulierend auf diese motorischen Bahnen ein: Während das Cerebellum vor allem die Lage und Koordination im Raum sowie die Stützmotorik beeinflusst, sind die Basalganglien grundlegend an der Initiierung, Durchführung und Richtung der Verhaltensantwort beteiligt (siehe Abbildung 1).

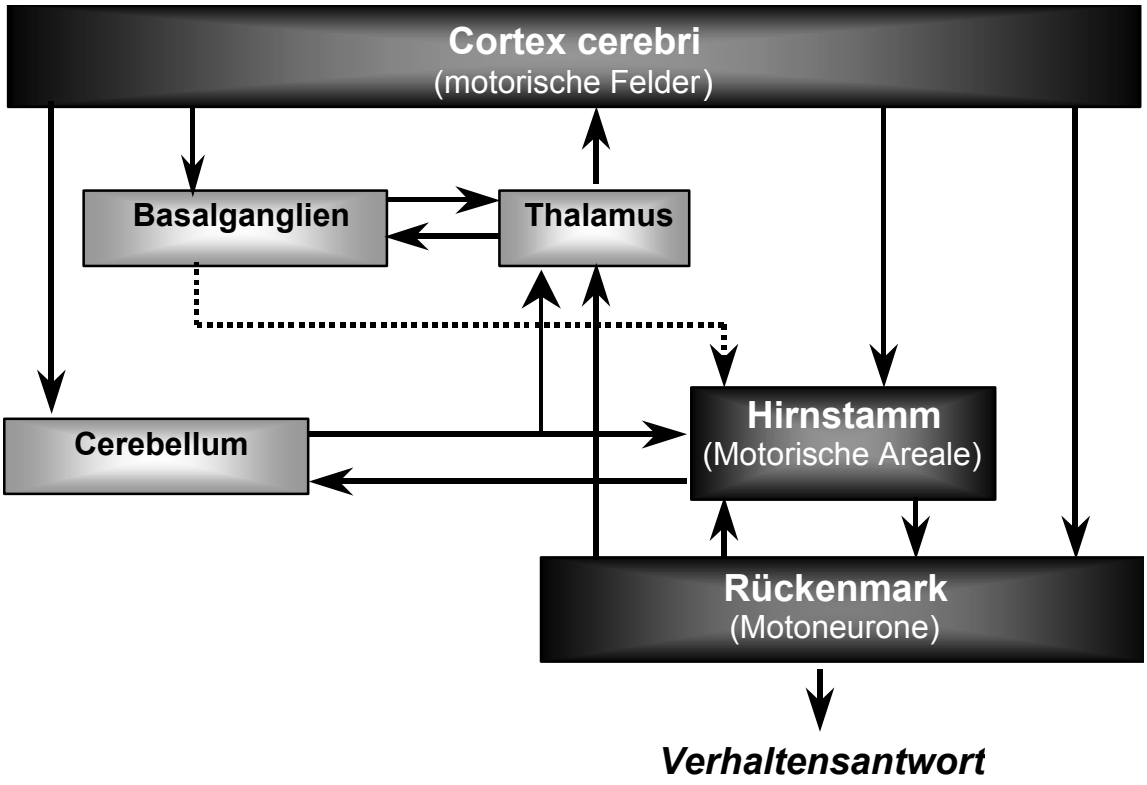

Abbildung 1: Aufbau der motorischen Systeme. Die motorischen Felder der Großhirnrinde können das Rückenmark sowohl direkt als auch indirekt über absteigende Systeme im Hirnstamm beeinflussen. Alle drei Instanzen der motorischen Systeme (dunkelgrau) stehen unter dem Einfluss zweier unabhängiger, subkortikaler Systeme (hellgrau): den Basalganglien und dem Cerebellum. Sowohl die Basalganglien als auch das Cerebellum wirken über Relaiskerne im Thalamus auf die Großhirnrinde ein. Ein weniger beachteter Ausgang verbindet die Basalganglien direkt mit dem Hirnstamm - ohne eine Rückprojektion zum Kortex (gestrichelter Pfeil).

1.1.1. Basalganglien und Verhaltensanpassung

Die Basalganglien bestehen aus subkortikalen Arealen im Vorderhirn und bilden über den Thalamus parallele, miteinander vernetzte Rückprojektionsschleifen. Diese Anordnung ermöglicht es den Basalganglien, Information aus dem Kortex zu modulieren und wieder an assoziierte Kortexregionen zu senden (Alexander et al., 1986; Joel and Weiner, 1994). Entsprechend den ihnen zugeordneten kortikalen Arealen (motorisch, dorsolateral-präfrontal, okulomotorisch, lateral-orbitofrontal und mediodorsal) werden bis heute fünf parallele Basalganglienschleifen

unterschieden (Alexander et al., 1986). Die Eingangsstruktur der dorsolateralen-präfrontalen (ventralen) Schleife ist der Nucleus accumbens (NAC). Er erhält glutamaterge limbische Information aus kortikalen Strukturen, z.B. aus dem präfrontalen Kortex (PFC), Amygdala oder Hippokampus (Heimer et al., 1991). Der NAC wird als Integrator zwischen den limbischen Eingängen, die emotionale und motivationale Information vermitteln, und dem motorischen Ausgang der Basalganglien verstanden (Mogenson, 1987; Pennartz et al., 1994; Groenewegen et al., 1996; Groenewegen et al., 1997). An der Integration der afferenten glutamatergen Signale ist mesoaccumbales Dopamin (DA) aus Neuronen des ventralen tegmentalen Areals (VTA) entscheidend beteiligt (Scheel-Krüger and Willner, 1991; Pennartz et al., 1994; Overton and Clark, 1997). Mesoaccumbales DA verstärkt oder hemmt die Verarbeitung von gleichzeitig auftretenden glutamatergen Signalen. Daher wird vermutet, dass DA im NAC ein Signal für bewertende und neuigkeitsvermittelnde Information darstellt (Salamone et al., 1997; Schmidt, 1998; Koch et al., 2000; Horvitz, 2002; Sesack et al., 2003). Die Strukturen die an diesen Prozessen beteiligt sind (limbisch-kortikale Regionen, dopaminerge Kerne und Basalganglien) werden als frontostriatales System zusammengefasst und könnten über den Mechanismus der DA/Glutamat (GLU)-Interaktion im NAC das neuroanatomische und neurochemische Substrat von Verhaltensanpassungsprozessen, wie der *response selection,* darstellen. DA/GLU-Interaktionen wären somit direkt verantwortlich für Prozesse, wie z.B. dem Umschalten (*switching*) zwischen konkurrierenden Bewegungsabläufen: z.B. ’Nahrungsaufnahme’ und ’Flucht’ (Redgrave et al., 1999; Gurney et al., 2001).

Eine mögliche Projektion über die die ventrale Basalganglienschleife Verhaltensanpassungsprozesse beeinflussen kann, ist die oben beschriebene Rückprojektionsschleife zum Kortex. Allerdings könnte Information auch direkt über Hirnstammstrukturen auf Motoneurone im Rückenmark umgeschaltet werden und so zu einer Verhaltensmodulation führen, ohne erneut den Kortex zu passieren (Winn, 1998) (siehe Abbildung 1).

1.1.2. Anatomische und neurochemische Betrachtung des PPTg

Der pedunculopontine tegmentale Nucleus (PPTg) ist Teil der motorischen Hirnstammareale (Abbildung 1) und liegt lateral zur Spitze des superioren cerebellaren Peduncels. Er umfasst einerseits große cholinerge Neurone, die als CH5-Neurone einen Teil der cholinergen Hirnstammgruppe bilden und andererseits kleinere nicht-cholinerge Neurone, die vorwiegend als glutamaterg, zum Teil auch GABAerg, beschrieben werden (Mesulam et al., 1983; Childs and Gale, 1984; Ford et al., 1995). Der PPTg wird durch Basalganglienkerne innerviert und könnte einen alternativen Ausgang für die Basalganglien darstellen (siehe 1.1.1.). Darüber hinaus liegt der PPTg in einer bemerkenswerten Position, da er nicht nur aus den Basalganglien innerviert wird, sondern auch Information aus einer Vielzahl weiterer Systeme erhält: So sind zusätzlich Afferenzen aus dem motorischen Kortex, den dopaminergen Mittelhirnarealen (VTA/Substantia Nigra pars compacta (SNc)) und den limbischen Strukturen, wie z.B. PFC, lateraler Hypothalamus und Amygdala beschrieben (Steininger et al., 1992; Semba and Fibiger, 1992). Als Hirnstammkern steht der PPTg zwar am unteren Ende der Hierarchie motorischer Systeme; er empfängt aber das gesamte Spektrum (motorischer und limbischer) verhaltensrelevanter Information. Dies ist umso bemerkenswerter, da der PPTg wiederum zu den höheren afferenten Strukturen zurückprojiziert. Dies könnte die anatomische Grundvoraussetzung für eine PPTg-vermittelte Beeinflussung von komplexem motiviertem Verhalten (z.B. Verhaltensanpassung) darstellen. (siehe Abbildung 2).

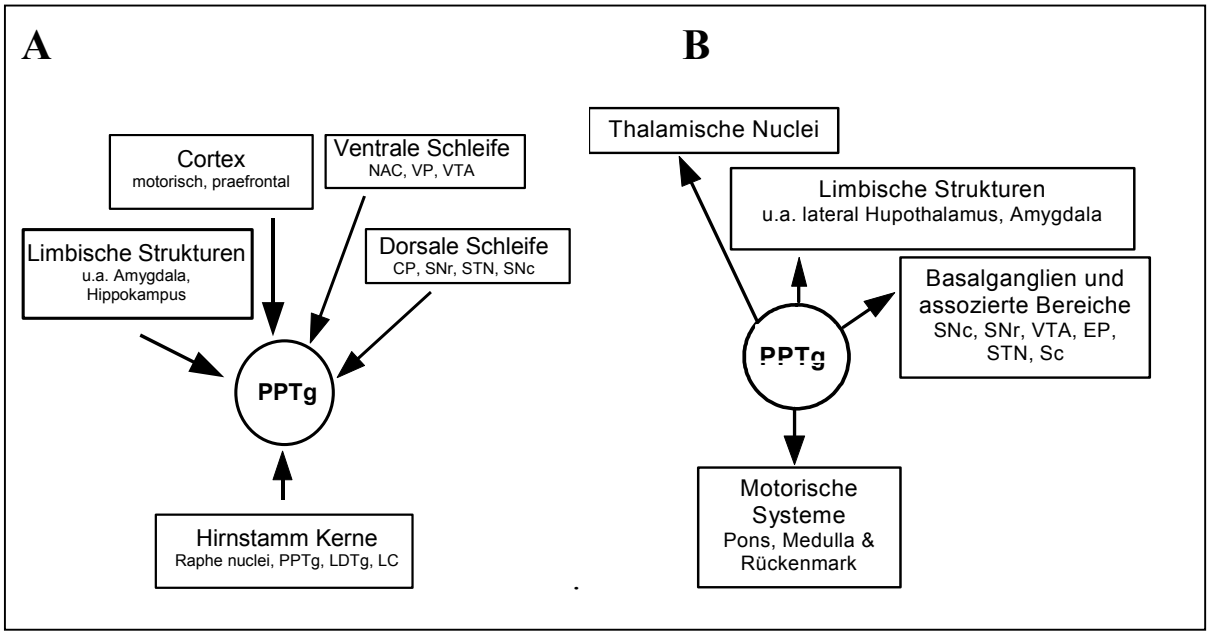

Abbildung 2: (A) Afferenzen und (B) Efferenzen des pedunculopontinen tegmentalen Nucleus (PPTg). CP: Caudate Putamen, EP: entopeduncularer Nucleus, LC: Locus Coeruleus, LDTg: lateraler tegmentaler Nucleus, NAC: Nucleus accumbens, Sc: superior Colliculus, SNc: Substantia nigra pars compacta, SNr: Substantia nigra pars reticulata, STN: subthalamischer Nucleus, VP: ventrales Pallidum, VTA: ventrales tegmentales Areal.

1.1.3. Funktionelle Betrachtung des PPTg

Das komplexe Verschaltungsmuster des PPTg deutet auf eine Beteiligung des PPTg an unterschiedlichen funktionellen Systemen hin. Grundsätzlich können dabei drei Systeme unterschieden werden:

(A) Der PPTg wird als zentraler Kern der mesencephalen lokomotorischen Region (*mesencephalic locomotor region*) angesehen. Dies ist eine Region in der lokale elektrische Stimulationen auch nach einer Transsektion zwischen Mamillarkörper und Colliculus, also nach der Abtrennung aller höheren Gehirnbereiche, koordinierte Lokomotionen auslösen (Shik et al., 1966). Die Einbindung des PPTg in die mesencephale motorische Region stellt eine direkte Verbindung des PPTg zur Steuerung von Lokomotion, also einfachem motorischem Verhalten, her.

(B) Des Weiteren ist der PPTg Teil des aufsteigenden retikulären Aktivierungssystems (*ascending reticular activation system* - ARAS). Dieses

System wird aus einem Band monoaminerger und cholinerger Kerne gebildet, welches auch als Hirnstamm-Oszillator bezeichnet wird. Dabei wird die rhythmische Neurotransmission aus dem ARAS zum Thalamus als Regulation von Schlaf-Wach-Zyklen und vom Erregungszustand angesehen. Psychologisch wird ein erhöhter Erregungszustand als Wachheit oder Bereitschaft zum Handeln verstanden: Somit bestimmt der Erregungszustand eines Tieres auch direkt dessen Aufmerksamkeit (Steriade and McCarley, 1990; Garcia-Rill E., 1991). Die Einbindung des PPTg in das ARAS impliziert, dass der PPTg funktionell an Erregungs- sowie Aufmerksamkeitsprozessen beteiligt sein könnte.

(C) Während eine Bedeutung des PPTg bei einfachen, sehr grundlegenden Verhaltensmechanismen (Motorik, Schlaf-Wach-Zyklen) gut etabliert ist, ist die Bedeutung des PPTg für komplexeres Verhalten, wie z.B. kognitive oder motivationale Prozesse, bisher spekulativ (als Übersicht siehe Steckler et al., 1994; Winn, 1998; Steiniger, 1999). Die oben beschriebenen anatomischen Verbindungen (siehe 1.1.2.) zu den höheren Strukturen, wie den Basalganglien und den limbischen Strukturen, legen eine solche Funktion allerdings nahe. Tatsächlich wurden bereits die Auswirkungen einer PPTg-Läsion auf komplexes Verhalten untersucht. Diese Studien zeigen, dass PPTg-lädierte Tiere bei der Durchführung komplexer Verhaltensmodelle, wie z.B. konditionierte Platzpräferenz, intrakraniale Selbststimulation, konditioniertes operantes Antwortverhalten und räumliche Lernaufgaben, beeinträchtigt sind. Da diese Modelle in der einen oder anderen Form kognitiv oder motivational gesteuertes Verhalten untersuchen, weisen diese Ergebnisse auf eine mögliche Funktion des PPTg bei komplexem Verhalten hin. Die zugrunde liegenden Mechanismen über die der PPTg komplexes Verhalten beeinflusst sind bisher jedoch nicht bekannt. Es werden aber Assoziations-, Belohnungs- oder Verhaltensanpassungsprozesse diskutiert. Insbesondere die Verhaltensanpassungsprozesse würden den PPTg mit DA/GLU-Interaktionen in frontostriatalen Systemen in Zusammenhang bringen. Eine systematische Untersuchung der verhaltensrelevanten, neurochemischen Interaktion des PPTg mit diesem System fehlt bisher jedoch.

1.2. Fragestellung

Gegenstand dieses Forschungsvorhabens war es, die Verhaltensrelevanz des PPTg unter Berücksichtigung seiner Verbindungen zu den Basalganglien zu untersuchen. Sowohl für den PPTg als auch für die Basalganglien sind verhaltensrelevante Funktionen bekannt. Während die ventralen Basalganglien vor allem motiviertes Verhalten modulieren, ist der PPTg, als Teil von Hirnstammsystemen (ARAS und MLR), an einfacheren, mehr grundlegenden Prozessen (z.B. Lokomotion und Schlaf-Wach-Zyklus) beteiligt. Über die Funktion der bestehenden, direkten (z.B. PPTg – NAC) und indirekten (z.B. über dopaminerge und limbische Systeme) reziproken Verbindungen des PPTg zu den ventralen Basalganglien, ist bisher jedoch nur wenig bekannt. Es wird spekuliert, dass der PPTg über diese Verbindungen an der Modulation von motiviertem Verhalten beteiligt ist. Deshalb wurde in dieser Arbeit untersucht, inwieweit die neurochemischen Interaktionen zwischen dem PPTg und den ventralen Basalganglien das Verhalten von Ratten beeinflussen.

Ausgehend von der großen Bedeutung des DA-Systems in basalganglienvermitteltem Verhalten wurde zunächst untersucht: (a) ob der PPTg dopaminerg innerviert wird; (b) ob es auf der Ebene des PPTg Interaktionen zwischen dem DA-System und den afferent zum PPTg gelegenen Transmittersystemen, d.h. GABAerge und glutamaterge Systeme, gibt und (c) welche Bedeutung die DA-Freisetzung im PPTg für motorisches Verhalten hat (Manuskript I).

Darüber hinaus kann aufgrund der anatomischen Verbindungen von einer Beteiligung des PPTg in komplexem motiviertem Verhalten ausgegangen werden. Läsionsstudien unterstützen dies (siehe oben). Deshalb wurde in den anschließenden Arbeiten (Manuskript II und III) die Auswirkungen einer PPTg-Inaktivierung (Ibotensäureläsion) auf exploratives und auf operantes Verhalten untersucht. Ausgehend von der Bedeutung der DA/GLU-Interaktion in den Basalganglien für motiviertes Verhalten wurde zudem untersucht, inwieweit es

Wechselwirkungen zwischen einer PPTg-Inaktivierung und glutamaterg, bzw. dopaminerg vermitteltem motiviertem Verhalten gibt.

Basierend auf den Erkenntnissen der vorausgegangenen Arbeiten wurde die Bedeutung der Interaktion zwischen dem PPTg, dem DA-System und dem GLU-System für die Ausführung eines operanten Verhaltens weitergehend charakterisiert. Hierfür wurde im Speziellen untersucht: (a) ob die GLU-Freisetzung im PPTg sowie die DA-Freisetzung im NAC zeitlich mit Veränderungen in motiviertem Verhalten korrelieren; (b) ob auf der Ebene des PPTg, über afferente GABAerge, sowie glutamaterge Systeme, die Transmitterfreisetzungen im PPTg und NAC moduliert werden; (c) welche Bedeutung die Transmitterfreisetzung im PPTg und NAC in motiviertem Verhalten haben; und (d) welche Bedeutungen die Interaktionen zwischen PPTg und DA-, bzw. GLU-Systemen für motiviertes Verhalten haben.

2. Zusammenfassungen der publizierten oder zur Publikation eingereichten Arbeiten

I. Glutamate and GABA modulate dopamine in the pedunculopontine tegmental nucleus

II. Effects of ibotenate pedunculopontine tegmental nucleus lesions on exploratory behavior in the open field

III. Performance of pedunculopontine tegmental nucleus lesioned rats in an operant discrimination task after pharmacological manipulations

IV. Different functions of pedunculopontine GABA- and glutamate-receptors in nucleus accumbens dopamine, pedunculopontine glutamate and operant discriminative behavior

2.1. Glutamate and GABA modulate dopamine in the pedunculopontine tegmental nucleus

Der PPTg ist der am weitesten caudal gelegene Kern, der Informationen aus den Basalganglien und limbischen Strukturen erhält und zusätzlich ausgeprägte Verbindungen zu motorischen Arealen im Hirnstamm und Rückenmark hat (Steininger et al., 1992; Semba and Fibiger, 1992). Darüber hinaus hat der PPTg über reziproke Verbindungen die Möglichkeit *Feedback* an die afferent liegenden Strukturen weiterzuleiten. Von besonderem Interesse sind hierbei die Verbindungen zwischen dem mesencephalen DA-System und dem PPTg, da DA entscheidend an verhaltensrelevanten Prozessen in frontostriatalen Systemen beteiligt ist. Es ist jedoch unklar, inwieweit die anatomischen Verbindungen zwischen PPTg und DA-System von funktioneller Bedeutung sind.

Deshalb wurde in dieser Arbeit die Interaktion des PPTg mit dem dopaminergen System pharmakologisch charakterisiert. Hierfür wurde untersucht, ob DA im PPTg freigesetzt wird und ob dies mit einer Veränderung des Verhaltens korreliert. Durch lokale Inhibition (GABA-Agonisten) sowie Stimulation (GLU-Agonisten) des PPTg wurde die Interaktion von DA mit afferenten Systemen des PPTg, wie den GABAergen Basalganglien oder den glutamatergen limbischen Regionen, näher charakterisiert. Die Wahl der Manipulation dieser Transmittersysteme im PPTg ermöglichte eine nähere Einordnung des PPTg in verhaltensrelevante frontostriatale Systeme.

Um die DA-Freisetzung im PPTg zu bestimmen, wurde die *in vivo* Mikrodialyse an frei beweglichen Ratten angewandt. Die quantitative Bestimmung von DA erfolgte über die Hochdruckflüssigkeitschromatographie (HPLC) in Kombination mit elektrochemischer Detektion. Zur pharmakologischen Charakterisierung der DA-Freisetzung wurden folgende Substanzen lokal in den PPTg infundiert: hochdosierte Kaliumchloridlösung, Amphetamin (DA-Wiederaufnahmehemmer), Baclofen ($GABA_B$-Agonist) sowie NMDA und AMPA (GLU-Agonisten). Die

Veränderungen der DA-Freisetzung wurden mit dem motorischen Verhalten der Ratte im *open field* korreliert.

Die Infusionen der präsynaptisch wirkenden Substanzen Kaliumchlorid und Amphetamin erhöhten die DA-Freisetzung im PPTg und demonstrierte somit, dass im PPTg freigesetztes DA neuronalen Ursprungs ist. Ebenso wurde die DA-Freisetzung im PPTg durch NMDA- und AMPA-Infusionen stimuliert und durch Baclofen-Infusion inhibiert. Allerdings führte nur die Inhibition zu einer leichten, durch Schnüffeln charakterisierten, Aktivierung der Tiere, die zeitlich jedoch nicht mit der reduzierten DA-Freisetzung korrelierte.

Wie bereits oben erwähnt, wird das DA-System als Vermittler verhaltensrelevanter Information in frontostriatalen Strukturen angesehen. Die Daten dieser Arbeit zeigen, dass auch der PPTg dopaminerge Information empfängt, die durch glutamaterge und GABAerge Afferenzen moduliert werden kann. Deshalb ist eine Funktion des PPTg in dopaminerg vermitteltem Verhalten wahrscheinlich. Ob DA im PPTg aber tatsächlich eine verhaltensrelevante Rolle hat, bleibt in dieser Arbeit offen, denn die geringen Verhaltensänderungen korrelierten zeitlich nicht mit der DA-Freisetzung und wurde nur im habituierten Tier untersucht; d.h. in Tieren, die an ihre Umgebung gewöhnt waren und nicht motiviert waren ein aktives Verhalten (z.B. Lokomotion) auszuführen. Allerdings deuten verhaltenspharmakologische Daten, die eine Beeinträchtigungen von motiviertem Verhalten durch PPTg-Inaktivierung zeigen (als Übersicht siehe Winn, 1998) sowie anatomische Studien, die eine Innervation des PPTg aus dem VTA und der SNc zeigen (Hokfeld et al., 1984; Steininger et al., 1992; Semba and Fibiger, 1992; Ichinohe et al., 2000) auf Funktionen sowohl des DA-Systems wie auch des PPTg bei motivationalen Prozessen hin. Es ist daher denkbar, dass DA im PPTg eine verhaltensrelevante Funktion bei der Ausführung von motiviertem Verhalten hat. Inwieweit DA im PPTg an diesen Prozessen beteiligt ist, kann vor dem Hintergrund der vorliegenden Daten hier nicht abschließend geklärt werden.

2.2. Effects of ibotenate pedunculopontine tegmental nucleus lesions on exploratory behavior in the open field

Der PPTg ist ein zentraler Kern der mesencephalen lokomotorischen Region (*mesencephalic locomotor region*) und als Teil dieser Region direkt an einfachem, grundlegendem Verhalten, wie z.B. Lokomotion beteiligt (Shik et al., 1966; Garcia-Rill E. and Skinner, 1987).). In der vorausgegangenen Arbeit (Manuskript I) lösten allerdings weder eine PPTg-Stimulation (GLU-Agonist) noch eine PPTg-Inhibition (GABA-Agonist) Lokomotion in habituierten Tieren aus. Auch andere Studien in habituierten PPTg-lädierten Tieren zeigten weder eine veränderte spontane noch eine veränderte substanzinduzierte (u.a. Amphetamine) Lokomotion (Swerdlow and Koob, 1987; Dellu et al., 1991; Olmstead and Franklin, 1994; Inglis et al., 1994a; Inglis et al., 1994b; Podhorna and Franklin, 1999; De Leonibus et al., 2001). Diese Studien deuten aber auf eine Funktion des PPTg bei motiviertem Verhalten hin. Unterstützt wird diese Annahme durch anatomische Verbindungen zwischen dem PPTg und dopaminergen und glutamatergen Strukturen, über die motiviertes Verhalten auf frontostriataler Ebene moduliert wird. Der PPTg könnte also über die Modulation dieser Strukturen motiviertes Verhalten beeinflussen.
Deshalb wurde in dieser Arbeit der Einfluss einer PPTg-Inaktivierung (Ibotensäureläsion) auf motiviertes Verhalten untersucht. Hierfür wurde den Tieren eine neue Umwelt (*open field*) präsentiert und die Motorik während der Exploration dieser neuen Umwelt analysiert. Die Exploration stellt eine Situation erhöhter Motivation dar, in der unbehandelte Tiere erhöhte motorische Aktivität zeigen, d.h. ein motiviertes Verhalten ausführen. Um die Bedeutung des PPTg sowie seiner Interaktionen mit dem DA-System und dem GLU-System für motiviertes Verhalten zu untersuchen, wurde die Exploration (Lokomotion, Aufrichten und Aktivität in der Mitte) von *Sham*- und PPTg-lädierten Tieren, die mit Saline, MK-801 (GLU-Antagonist) oder Amphetamin (DA-Agonist) behandelt wurden, verglichen.

Die PPTg-Läsion hatte keine Effekte auf die spontane Exploration. Sie verstärkte aber die MK-801-induzierte Lokomotion und blockierte die Amphetamin-induzierte Erhöhung der Lokomotion, des Aufrichtens und der 'Aktivität in der Mitte des *open field',* d.h. des Explorationsverhalten insgesamt.
Diese Studie zeigt, dass eine PPTg-Läsion spontanes motiviert-motorisches Verhalten, in diesem Fall die Exploration nicht beeinflusste. Dieses Ergebnis wird durch die oben genannten Studien bestätigt, die keine PPTg-läsions-abhängige Beeinträchtigung von nicht-motiviert-motorischem Verhalten, d.h. motorisches Verhalten in habituierten Tieren, gefunden haben. Darüber hinaus zeigt die vorliegende Arbeit, dass eine PPTg-Läsion nur dann motiviert-motorisches Verhalten beeinflusst, wenn gleichzeitig entweder das dopaminerge oder das glutamaterge System stimuliert wurde. Dieses Ergebnis steht im Gegensatz zu den Studien, in denen PPTg-lädierte Tiere auch nach einer Stimulation des dopaminergen Systems keine Beeinträchtigung von nicht-motiviert-motorischem Verhalten zeigten (Olmstead and Franklin, 1994; Inglis et al., 1994b). Es scheint also eine Interaktion zwischen PPTg-Läsion und dem Motivationsaspekt zu geben (motiviert-motorisches Verhalten im Gegensatz zu nicht-motiviert-motorischem Verhalten), denn unter der Voraussetzunge pharmakologischer Stimulation beeinflusst eine PPTg-Läsion selektiv motiviertes, aber nicht nicht-motiviertes Verhalten. Unterstützt wird diese Hypothese durch Befunde die zeigen, dass eine PPTg-Läsion auch motiviertes Verhalten blockiert, welches nur einen geringen motorischen Anteil hat: In diesen Studien blockierte die PPTg-Läsion die Motivation eine Umgebung aufzusuchen, die zuvor mit einem positiven Stimulus (Futter, Opioide oder Amphetamine) gepaart wurde (konditionierte Platzpräferenz) (Bechara and van-der-Kooy, 1989; Olmstead and Franklin, 1994; Bechara et al., 1998).

Zusammenfassend zeigen die Daten, dass über den PPTg induzierte Verhaltensmodulationen vom motivationalen Status des Tieres abhängen. Aufgrund der hier gezeigten Interaktionen mit dem DA- und GLU-System, die

ihrerseits vornehmlich über die Basalganglien Einfluss auf Verhalten nehmen, erscheint eine Interaktion des PPTg mit den Basalganglien, möglicherweise über den NAC, für die Effekte auf motivationales Verhalten verantwortlich zu sein.

2.3. Performance of pedunculopontine tegmental nucleus-lesioned rats in an operant discrimination task after pharmacological manipulations

DA/GLU-Interaktionen, vor allem im NAC, wird eine Schlüsselfunktion bei der motivationalen Verhaltensanpassung zugerechnet. DA wird dabei als Signal für bewertende und neuigkeitsvermittelnde Information verstanden, das gleichzeitig auftretende glutamaterge Signale verstärkt oder hemmt. Die glutamatergen Signale aus limbisch-kortikalen Regionen repräsentieren dabei vor allem motivationale Information (Salamone et al., 1997; Schmidt, 1998; Koch et al., 2000; Tzschentke and Schmidt, 2000; Horvitz, 2002; Sesack et al., 2003).

Der PPTg ist mit Strukturen verbunden, die an der striatalen DA/GLU-Interaktion beteiligt sind (Hallanger and Wainer, 1988; Heimer et al., 1991; Steininger et al., 1992; Semba and Fibiger, 1992). Insbesondere stellt der PPTg neben den kortikalen Regionen eine weitere exzitatorische Afferenz der dopaminergen Mittelhirnneurone dar (Jackson and Crossman, 1983; Kalivas, 1993) und könnte so motiviertes Verhalten beeinflussen. In diesem Zusammenhang wurde in dem vorausgegangen Manuskript (II) gezeigt, dass eine PPTg-Läsion motiviert-motorisches Verhalten (Exploration) beeinflusste. Dieser Effekt war allerdings nur vorhanden, wenn die Tiere zusätzlich über das DA-System bzw. GLU-System stimuliert wurden. Auch andere Studien zeigen eine Beeinträchtigung von motiviertem Verhalten nach PPTg-Läsion. Über die Defizite, die zu diesen Verhaltensbeeinträchtigungen führen kann aber nur spekuliert werden (als Übersicht siehe Winn, 1998). Der zuvor in Manuskript II gezeigte Zusammenhang zwischen PPTg-Läsion, DA-System sowie GLU-System bei der Beeinflussung von motiviertem Verhalten (siehe Manuskript II) deutet aber auf Defizite in der Verhaltensanpassung hin. Denn die Verhaltensanpassung wird über eine Beeinflussung striataler DA/GLU-Interaktionen moduliert (siehe oben). Deshalb wurde in der vorliegenden Arbeit untersucht ob: (a) ein komplexes, motiviertes Verhalten, bei dem verstärkt die Verhaltensanpassung benötigt wird, direkt vom PPTg abhängt; und (b) welche Bedeutung einer eventuellen Wechselwirkung zwischen dem PPTg und dem DA-

sowie GLU-System bei der Ausführung dieses komplexen motivierten Verhaltens zukommt.

Hierfür wurde der operante Diskriminierungstest (ODT) entwickelt: In diesem Test muss eine futterbelohnte Taste von einer unbelohnten Taste unterschieden werden. Durch das andauernde, aber zufällige Wechseln der Tasten (belohnt – unbelohnt, links – rechts), fordert diese Aufgabe eine immer neue Verhaltensanpassung (z.B. "linke Taste drücken und rechte Taste ignorieren" wird abgelöst durch "linke Taste ignorieren und rechte Taste drücken"). *Sham*- und PPTg-lädierte Tiere wurden mit Saline, MK-801 (GLU-Antagonist) oder Amphetamin (DA-Agonist) behandelt und anschließend deren Leistung im ODT (*response*[1]-Rate und *response*-Genauigkeit) verglichen.

PPTg-Läsionen reduzierten nachhaltig die *response*-Rate und die *response*-Genauigkeit. Ebenso reduzierte MK-801 die *response*-Rate und die *response*-Genauigkeit, wobei dieser Effekt bei PPTg-, gegenüber *Sham*-lädierten Tieren ausgeprägter war. Amphetamin hatte weder auf die *response*-Rate noch auf die *response*-Genauigkeit von *Sham*-lädierten Tieren Auswirkungen. Allerdings reduzierte es die *response*-Genauigkeit, nicht aber die *response*-Rate von PPTg-lädierten Tieren.

Die Daten dieser Studie legen nahe, dass der PPTg komplexes, motiviertes Verhalten beeinflusst. Einerseits bestätigt diese Studie frühere Befunde die zeigen, dass PPTg-lädierte Tiere zwar die Assoziation "Tastendruck-führt-zur-Präsentation-eines-konditionierten-Verstärkers" (z.B. Licht) abrufen, dabei aber nicht zwischen der belohnten und der unbelohnten Taste unterscheiden (Inglis et al., 1994b; Inglis et al., 2000). Andererseits erweitert diese Studie die Befunde, denn ein solches Diskriminierungsdefizit wurde hier auch für die Präsentation eines primären Verstärkers (Futter) gefunden. Zusätzlich wurde gezeigt, dass diese Effekte von der Funktion des DA-Systems sowie des GLU-Systems abhängen.

[1] response: (Psychol. Sprachw.) durch einen Reiz ausgelöstes, bestimmtes Verhalten (Duden (5), 7. Auflage)

Über direkte anatomische Verbindungen zwischen dem PPTg und den DA- sowie GLU-Systemen könnte der PPTg komplexes motiviertes Verhalten beeinflussen. Wie oben beschrieben, ist die DA/GLU-Interaktion im NAC an motivationalen Verhaltensanpassungsprozessen beteiligt. PPTg-Läsionen könnten Defizite in diesen Prozessen auslösen, die zu den hier gefundenen Verhaltensdefiziten führen. Tatsächlich führen auch NAC-Läsionen zu Defiziten in operanten Tests, die den Defiziten nach PPTg-Läsion gleichen (Balleine and Killcross, 1994; Salamone et al., 1997; Schmidt, 1998; Koch et al., 2000; Tzschentke and Schmidt, 2000; Horvitz, 2002; Sesack et al., 2003).

Zusammenfassend resultieren die in dieser Studie gefundenen Defizite wahrscheinlich aus einer veränderten Verhaltensanpassung, die auf eine Modulation der DA/GLU-Interaktion im NAC zurückzuführen ist.

2.4. Different functions of pedunculopontine GABA- and glutamate-receptors in nucleus accumbens dopamine, pedunculopontine glutamate and operant discriminative behavior

Der PPTg interagiert anatomisch und funktionell mit den Basalganglien. In diesem Zusammenhang wurde in den vorausgegangenen Arbeiten gezeigt, dass: (a) der PPTg Information aus dem DA-System erhält und DA im PPTg über das GLU- und das GABA-System moduliert wird (Manuskript I); (b) Verhaltensmodulationen, die über den PPTg ausgelöst werden, vom motivationalen Status des Tieres abhängen (Manuskript II); (c) ein intakter PPTg insbesondere für die korrekte Ausführung von komplexem, motiviertem Verhalten (ODT) notwendig ist (Manuskript III) und (d) es Wechselwirkungen zwischen PPTg-Inaktivierung und Manipulationen des DA-Systems sowie des GLU-Systems bei der Ausführung von motiviertem Verhalten gibt (Manuskripte II und III).

Diese Studien deuten darauf hin, dass der PPTg über eine Modulation der DA/GLU-Interaktion im NAC Verhaltensanpassungsprozesse verändert und somit die Ausführung von motiviertem Verhalten beeinflusst.

Um diesen Aspekt näher zu untersuchen, wurde in Tieren, die den ODT ausführten, die DA-Freisetzung im NAC (mesoaccumbales DA) sowie die GLU-Freisetzung im PPTg gemessen. Um die Relevanz des PPTg dabei näher zu charakterisieren, wurde der PPTg zusätzlich inhibiert (über Baclofen) bzw. stimuliert (über AMPA).

Die GABA-abhängige PPTg-Inhibition reduzierte die *response*-Rate und die *response*-Genauigkeit im ODT, wohingegen die AMPA-abhängige PPTg-Stimulation nur die *response*-Rate reduzierte. Beide Behandlungen blockierten den ODT-induzierten Anstieg der DA-Freisetzung im NAC, gleichzeitig wurde nach der PPTg-Stimulation eine erhöhte GLU-Freisetzung im PPTg gemessen.

Auffällig ist, dass die mesoaccumbale DA-Freisetzung sowohl nach einer PPTg-Stimulation (GLU-Agonist) als auch nach einer PPTg-Inhibition (GABA-Agonist) geblockt war. Die Tatsache, dass unterschiedliche PPTg-Manipulationen die gleichen DA-Effekten auslösen, lässt darauf schließen, dass das GLU- und das

GABA-System unterschiedliche Efferenzen des PPTg, d.h. unterschiedliche Schaltkreise ansprechen. Während die GABA-Effekte eine direkte Inhibition der exzitatorischen PPTg-Neurone, die zum VTA projizieren, wahrscheinlich machen (Kalivas, 1993; Lavoie and Parent, 1994; Oakman et al., 1995; Parent et al., 1999), deuten die AMPA-Effekte auf *long-loop*-Verbindungen hin, die den PFC mit einbeziehen (Tzschentke and Schmidt, 2000; Sesack et al., 2003). Die direkte, glutamaterge Projektion vom PFC zum PPTg (Tzschentke and Schmidt, 2000; Sesack et al., 2003) und die AMPA-induzierte Erhöhung der GLU-Freisetzung im PPTg unterstützen diese *long-loop*-Hypothese. Zusätzlich wurden in Diskriminierungsaufgaben Verhaltensdefizite nach PFC-Läsion gefunden, die den hier dargestellten Verhaltensdefiziten nach PPTg-Läsion gleichen (Muir et al., 1996; Baunez and Robbins, 1997).

Die mesoaccumbale DA-Freisetzung korrelierte mit der *response*-Rate, denn eine hohe *response*-Rate in Saline behandelten Tieren führte zu einer hohen DA-Freisetzung, wohingegen eine geringe *response*-Rate nach PPTg-Manipulation (Stimulation und Inhibition) zu einer Blockade der DA-Freisetzung führte. Die durch eine PPTg-Stimulation ausgelöste, erhöhte GLU-Freisetzung im PPTg könnte dahingegen ein Kompensationssignal für die *response*-Genauigkeit darstellen. Denn die *response*-Genauigkeit war nur nach PPTg-Inhibition reduziert, dies führte jedoch nicht zu einer erhöhten GLU-Freisetzung im PPTg.

Zusammenfassend unterstützen diese Daten die Annahme, dass der PPTg komplexes, motiviertes Verhalten beeinflusst indem er DA- und GLU-Systeme auf frontostriataler Ebene moduliert.

3. Manuskripte der publizierten oder zur Publikation eingereichten Arbeiten

I

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Glutamate and GABA modulate dopamine in the pedunculopontine tegmental nucleus

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Abstract

The pedunculopontine tegmental nucleus (PPTg) has an important anatomical position connecting basal ganglia and limbic systems with motor execution structures in the pons and spinal cord. It receives glutamatergic and GABAergic input and has additional reciprocal connections with mesencephalic dopaminergic neurons, suggesting that the PPTg plays a key role in fronto-striatal information processing. *In vivo* microdialysis in freely moving rats, in combination with behavioral analysis, was used in this study to investigate whether the dopaminergic input can be modulated at the level of the PPTg via N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-isoxazole-4-prpionic acid (AMPA) or $GABA_B$ receptors.

Stimulation of the $GABA_B$ receptor decreased dopamine release in the PPTg while that of the AMPA and NMDA receptors increased it. A time-related comparison of the effects of NMDA (0.75 and 1 mM) and AMPA (50 and 25 μM) revealed a more long-lasting effect after AMPA stimulation than after NMDA. However, only the infusion of the $GABA_B$ receptor agonist baclofen (100 and 200 μM) stimulated stereotyped behavior (e.g. sniffing, digging or head movements) and contra lateral circling.

This study clearly demonstrates that GABAergic as well as glutamatergic terminals in the PPTg are critically involved in the modulation of the dopamine system. Moreover, a decrease in PPTg dopamine via $GABA_B$ receptor stimulation seems to be behaviorally relevant.

Keywords: basal ganglia rats in vivo microdialysis locomotor behavior behavior dopamine glutamate pedunculopontine tegmental nucleus mesopontine tegmentum

Introduction

The basal ganglia (BG) are involved in the control of motor behavior, but there is also substantial evidence for a role in reward, emotional and cognitive functions (Schmidt and Kretschmer 1997; Schultz et al. 1997; Graybiel 1997; Kalivas and Nakamura 1999). Anatomically they consist of subcortical areas (striatum and nucleus accumbens (NAC), substantia nigra reticulata (SNr), globus pallidus (GP) and ventral pallidum (VP)) that form re-entry loops with the cortex (Alexander et al. 1986). Basically, two major afferent transmitter systems interact on the BG level: the dopaminergic and the glutamatergic (Pennartz et al. 1994; Schmidt 1998). Dopamine (DA) from the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) plays a key role in the processes ascribed to the BG. DA is involved in response initiation and selection (i.e., facilitation or inhibition of behavior) and drug-induced reward (Schultz et al. 1995; Overton and Clarke 1997). This becomes obvious under pathological conditions; degeneration of dopaminergic neurons found in Parkinson's disease causes akinesia and rigidity; stimulation of DA release induced by drugs of abuse, i.e., cocaine and amphetamine, causes addiction as well as psychotic behavior, a dysfunction of response selection. Glutamate (GLU) from cortex, thalamus and limbic structures has been identified as the driving force of the BG; it innervates all BG nuclei and interacts with N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-isoxazole-4-prpionic acid (AMPA) and metabotropic GLU receptors (Albin et al. 1992).

Research mainly focuses on modulatory functions of this cortico-striato-thalamo-cortical loop. However, besides this re-entry loop there is a direct projection from BG nuclei to the brain stem. This projection terminates in the mesopontine tegmentum that consists of the pedunculopontine tegmental nucleus (PPTg) and the laterodorsal tegmental nucleus (LDTg). This region receives direct input from virtually all BG nuclei (NAC, SNr, GP, VP and subthalamic nucleus (STN)) motor cortex, limbic areas and dopaminergic midbrain neurons (Cornwall et al. 1990;

Heimer et al. 1991; Steininger et al. 1992; Semba and Fibiger 1992; Matsumura et al. 2000, Zahm et al. 2001), and is directly connected to motor neurons in the spinal cord (Rye et al. 1988; Skinner et al. 1990; Garcia-Rill et al. 2001). Thus, the mesopontine tegmentum is in a position to act as an output station of BG (Winn 1998). Furthermore, it sends ascending projections containing GLU and acetylcholine (ACh) to BG (NAC, SNr, GP and STN), lateral hypothalamus, basal forebrain, thalamus and dopaminergic midbrain (Jackson and Crossman 1983; Hallanger and Wainer 1988; Cornwall et al. 1990; Lavoie and Parent 1994a). Consequently, PPTg and LDTg can be understood as the last nuclei in behavior-relevant circuits that receive the whole spectrum of information from BG and, in turn, modulates behavior through BG or dopaminergic midbrain.

In fact, behavioral studies indicated a modulatory function of the PPTg in locomotion (Brudzynski and Mogenson 1985; Milner and Mogenson 1988; Mogenson et al. 1989; Bechara and van der Kooy 1992) as well as in stimulus reward association (Inglis et al. 2000; Satorra-Marín et al. 2001), but the exact role of the PPTg is far from clear (Steckler et al. 1994; Inglis and Winn 1995; Winn 1998). Since anatomical data reveal direct input from midbrain dopaminergic neurons, behavior modulated by the PPTg might be controlled by DA transmission. However, there is a substantial lack of knowledge concerning neurochemical interactions in the PPTg. Therefore, we used *in vivo* microdialysis (Ungerstedt et al. 1982) in freely moving rats to study pharmacological characteristics of DA release in the PPTg and to correlate the neurochemical outcome with behavior.

Materials and Methods

Animals

All experiments were carried out in male Sprague Dawley rats (Charles River, Sulzfeld, Germany) weighing 220 –260 g. Before surgery the rats were housed in groups of six to eight in laboratory cages (Pereg; Techniplast 95x44x21 cm) under standard conditions (temperature 22 ± 3°C; light from 0600 to 1800 hours). They were fed once a day with standard laboratory chow (12g/rat per day). Tap water was available ad libitum. After surgery the rats were housed in single cages (30x45x30cm) under the same conditions.

Experiments were done in accordance with the ethical guidelines regarding the care and use of animals and were approved by the local council of animal care (ZP1/00).

Surgery, microdialysis and behavioral analysis

Rats were anaesthetized with chloral hydrate (350mg/kg i.p.) and placed in a stereotaxic frame. A guide cannula (CMA microdialysis; Semrau AG, Sprockhövel, Germany) was unilaterally implanted into the PPTg (AP -7.8 mm, L -1.6 mm, V -6.2 mm to bregma, according to Paxinos and Watson 1998), using standard stereotaxic procedures described earlier (Kretschmer 1999).

After a minimum recovery period of 72 h, a microdialysis probe (CMA/12, CMA microdialysis) with an active membrane length of 2 mm and a diameter of 0.5 mm was gently inserted into the PPTg and perfused with artificial cerebrospinal fluid (aCSF) containing 147 mM NaCl, 2.5 mM KCl, 1.3 mM $CaCl_2$ and 0.9 mM $MgCl_2$ with a pH of 6.8 - 7.4 and a flow rate of 3 µl/min.

After a 3 h equilibrium period under aCSF, samples were collected every 20 min. Three baseline samples were taken, followed by three samples under drug condition and a further three samples under aCSF.

In a first series of experiments 100 mM KCl (Fluka, Neu-Ulm, Germany) or 1 µM of the DA agonist DL-amphetamine sulfate (Th. Geyer GMBH, Stuttgart,

Germany) was infused, to evaluate the neural origin of the measured DA and metabolites.

In a second series of experiments the glutamate receptor agonist AMPA (25 or 50 µM; Biotrend RBI, Köln, Germany) and NMDA (0.75 or 1 mM; Sigma, Deisenhofen, Germany) or the $GABA_B$ agonist (±) baclofen (100 or 200 µM; Sigma) were infused.

Osmolarity of 100 mM KCl solution was obtained by reducing NaCl content. The experiments with amphetamine (see above) were carried out as an extension of other experiments; therefore only two baseline values were taken.

After the experiments, the brain of each rat was removed and transferred to 4% Para formaldehyde. The localization of the probes was examined in cresyl violet stained frontal brain sections (30 µM).

NMDA was dissolved in a minimum amount of NaOH and diluted with aCSF to the final concentration. All other drugs were dissolved in aCSF. The pH of drug solutions was adjusted according to that of the aCSF.

Biochemical analysis

DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as 5-hydroxyindole acetic acid (5-HIAA) were immediately analyzed using reverse phase high-performance liquid chromatography (Bischoff, Leonberg, Germany) with electrochemical detection (ESA, Bischoff) as described earlier (Kretschmer 1999). Briefly, a HPLC pump (Bischoff) connected to a Prontosil 53 x 3 column (Bischoff), containing a mobile phase of 60 mM NaH_2PO_4, 0.1 mM EDTA, 0.2 mM octanesulfonic acid and 10% methanol (pH 3.73), were used. A detection limit of 0.05-0.06 nM was routinely achieved.

Behavioral analysis

During the experiments, rats were attached to a freely moving setup (CMA microdialysis) and placed in an open field (37 x 47 cm) that was subdivided into 2 x 3 squares. A video camera was fixed to the open field. Observation started when

drugs were infused and lasted until the end of the experiments. 18 min of a 20 min sampling period (to reduce artifacts during vial exchange) were analyzed for motor activity by quantifying the following parameters, (1) counts of line crossing (locomotion), (2) counts of full 360° body turns (circling), (3) active sitting (stereotyped behavior in minutes; e.g. sniffing, digging, head movements), (4) inactive sitting (min) and (5) grooming (min).

Presentation and statistical analysis

All neurochemical data were presented as differences relative to baseline values, which were defined as 100%. These data were assessed by repeated measures one-way analysis of variance (ANOVA) followed by the Fischer´s LSD-(protected t) test, if appropriate. The comparison of data obtained at different doses of substances was done using a two-way ANOVA followed by the Fischer´s LSD-(protected-t)-test. The level of significance was set at $p < 0.05$.

Results

Histology

As shown in Fig. 1, tracks of microdialysis probes were localized in the PPTg. Data from rats where probes were located outside of the PPTg were excluded from analysis. After NMDA or AMPA infusion into the PPTg no cell loss has been observed around the probe.

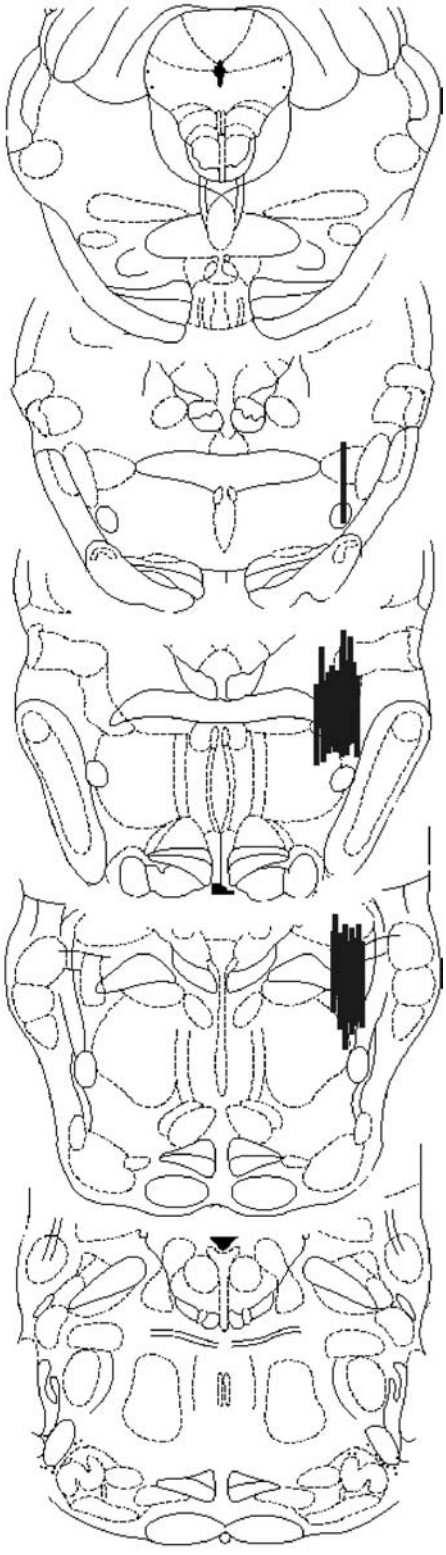

Fig. 1 Coronal sections through the rat brainstem (modified after Paxinos and Watson, 1998) showing the localization of microdialysis probes (black rectangles) in the pedunculopontine tegmental nucleus. Top section 6.8 mm and bottom 8.8 mm caudal of bregma.

Microdialysis

Under basal conditions the mean level of DA, DOPAC and HVA in the PPTg were 0.077 ± 0.006, 7.08 ± 0.36 and 8.08 ± 0.45 nM, respectively.

Table 1: Effects of local drug administration into the pedunculopontine tegmental nucleus (PPTg) on DA, DOPAC, HVA and 5-HIAA levels in the PPTg.

	DA	DOPAC	HVA	HIAA
	% of basal mean ± SEM			
100 mM KCl	1643 ± 215**	190 ± 23**	147 ± 9**	65 ± 5**
1 µM amphetamine	355 ± 83**	62 ± 3**	67 ± 3**	119 ± 17
0.75 mM NMDA	141 ± 14	205 ± 38**	152 ± 20**	71 ± 4**
1 mM NMDA	400 ± 94**	206 ± 18**	197 ± 23**	65 ± 7**
25 µM AMPA	378 ± 55**	313 ± 43**	196 ± 12**	82 ± 0.9**
50 µM AMPA	429 ± 74**	368 ± 51**	334 ± 55**	128 ± 10**
100 µM baclofen	58 ± 16*	78± 3	77 ± 12	78 ± 15
200 µM baclofen	76 ± 8**	112 ± 11	84 ± 9	86 ± 5**

The concentration of DA, DOPAC, HVA and 5-HIAA in the dialysate of the PPTg were determined after local drug administration into the PPTg. The maximum effects observed during or after the treatment are shown as percentage (mean ± SEM) of the basal mean level.*$p < 0.05$, **$p < 0.01$ as significant differences of drug administration data versus basal mean levels: data were analyzed by repeated measures one-way ANOVA followed by the Fischer´s LSD (protected t) test, if appropriate.

KCl infusion

Local infusion of 100 mM KCl-solution immediately increased the extracellular DA levels (Table 1, Fig. 2). This increase lasted until the end of the stimulation and was accompanied by a delayed increase of the levels of DA metabolites, DOPAC and HVA (Table 1).

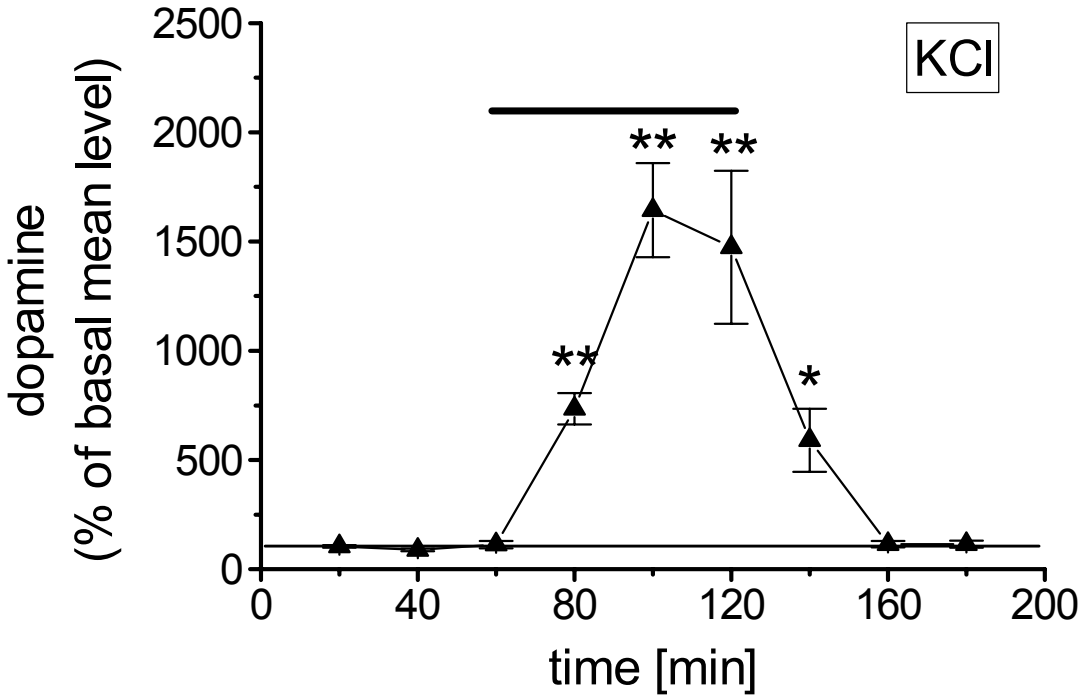

Fig. 2. Effects of KCl infusion (100 mM; n = 5) into the pedunculopontine tegmental nucleus for 1 h (indicated by the bar) on the extracellular concentration of dopamine in the microdialysis samples. Data are presented as percentage of the basal mean level ± SEM. Data were analyzed by repeated measures one-way ANOVA followed by the Fischer´s LSD-(protected t) test, if appropriate. *p < 0.05 and **p < 0.01, significant differences for 100 mM KCl data versus basal mean levels.

DL-amphetamine infusion

Local infusion of 1 µM DL-amphetamine increased DA levels continuously, reaching a maximum at the end of drug infusion, while DOPAC and HVA levels were decreased (Table 1, Fig. 3).

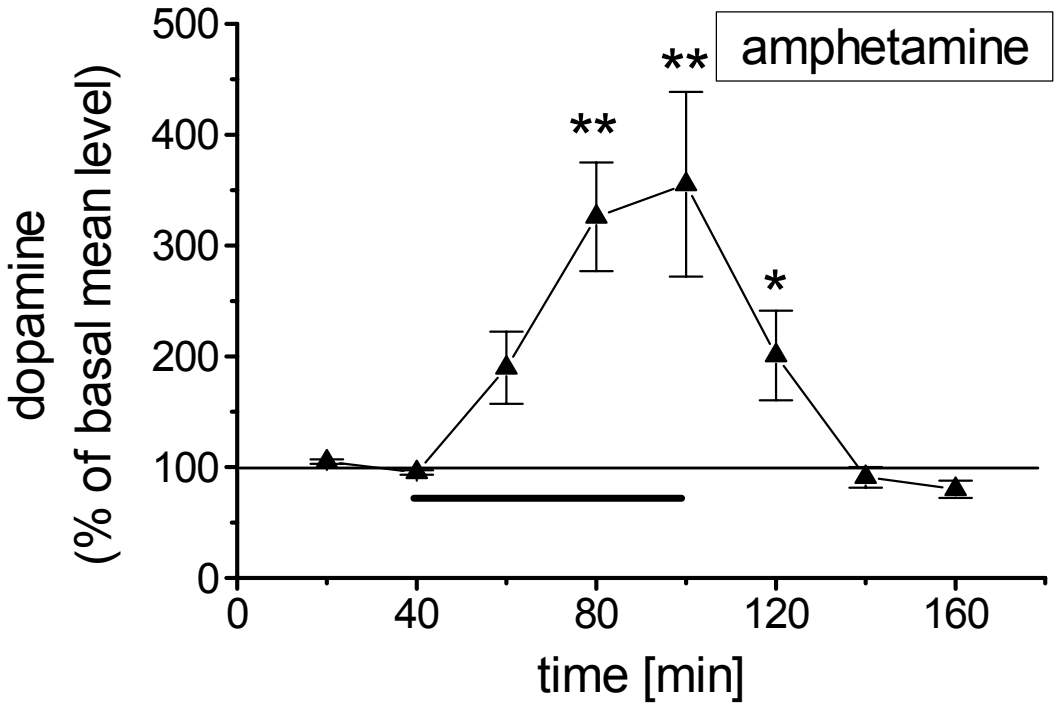

Fig. 3 Effects of amphetamine infusion (1 µM; n = 6) into the pedunculopontine tegmental nucleus for 1 h (indicated by bar) on the extracellular concentration of dopamine in the microdialysis samples. Data are presented as percentage of the basal mean level ± SEM. Data were analyzed by repeated measures one-way ANOVA followed by the Fischer´s LSD-(protected t) test, if appropriate. *$p < 0.05$, **$p < 0.01$, significant differences for 1 µM amphetamine data versus basal mean levels.

NMDA infusion

The high concentration of NMDA (1 mM), but not the low concentration (0.75 mM) enhanced extracellular DA levels (Table 1, Fig. 4). DOPAC levels were enhanced by both doses of NMDA. Furthermore, HVA levels were increased after a delay, but in a dose-related manner (Table 1).

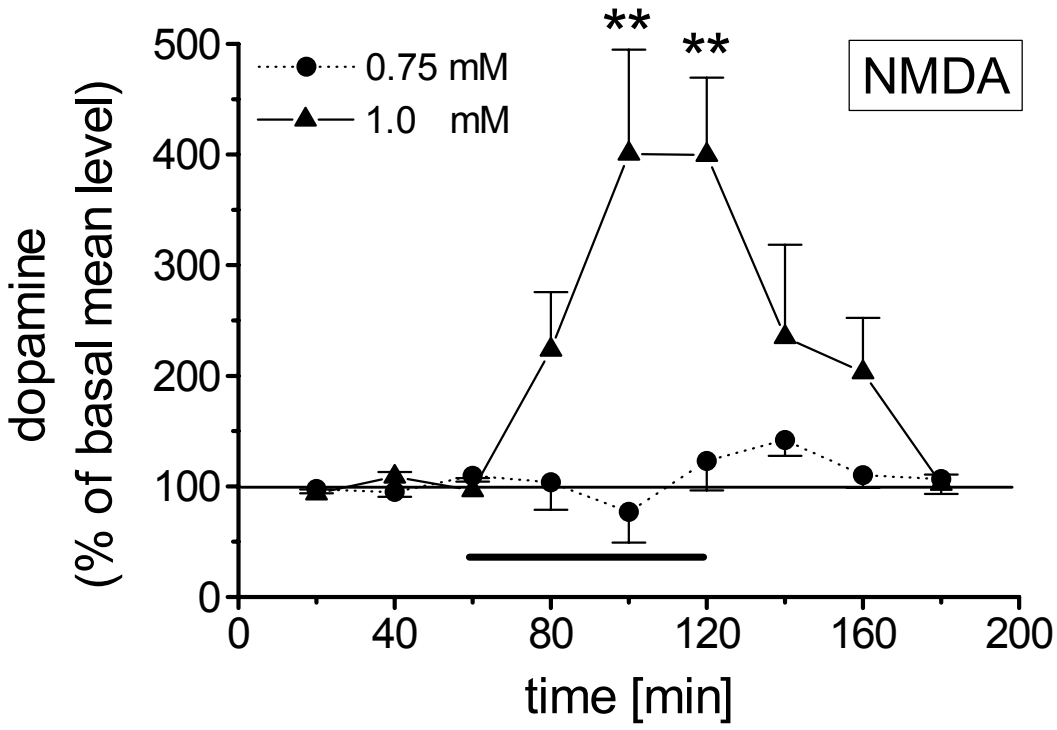

Fig. 4 Effects of NMDA infusion (0.75 mM, circles, n = 4 and 1 mM, triangles, n = 5) into the pedunculopontine tegmental nucleus for 1 h (indicated by bar) on the extracellular concentration of dopamine in the microdialysis samples. Data are presented as percentage of the basal mean level ± SEM. Data were analyzed by repeated measures one-way ANOVA followed by the Fischer´s LSD-(protected t) test, if appropriate. **p < 0.01, significant differences for 1 mM NMDA data versus basal mean levels, respectively.

AMPA infusion

AMPA perfused in concentrations of 25 and 50 µM enhanced DA levels in a dose-related manner (Table 1, Fig. 5). This increase was followed by a delayed and dose-related increase of DA metabolites levels, first DOPAC then HVA (Table 1).
A time related comparison of the neurochemical effects of 1 mM NMDA and 50 µM AMPA revealed a more long-lasting increase of DA levels after AMPA stimulation ($p < 0.001$; ANOVA).

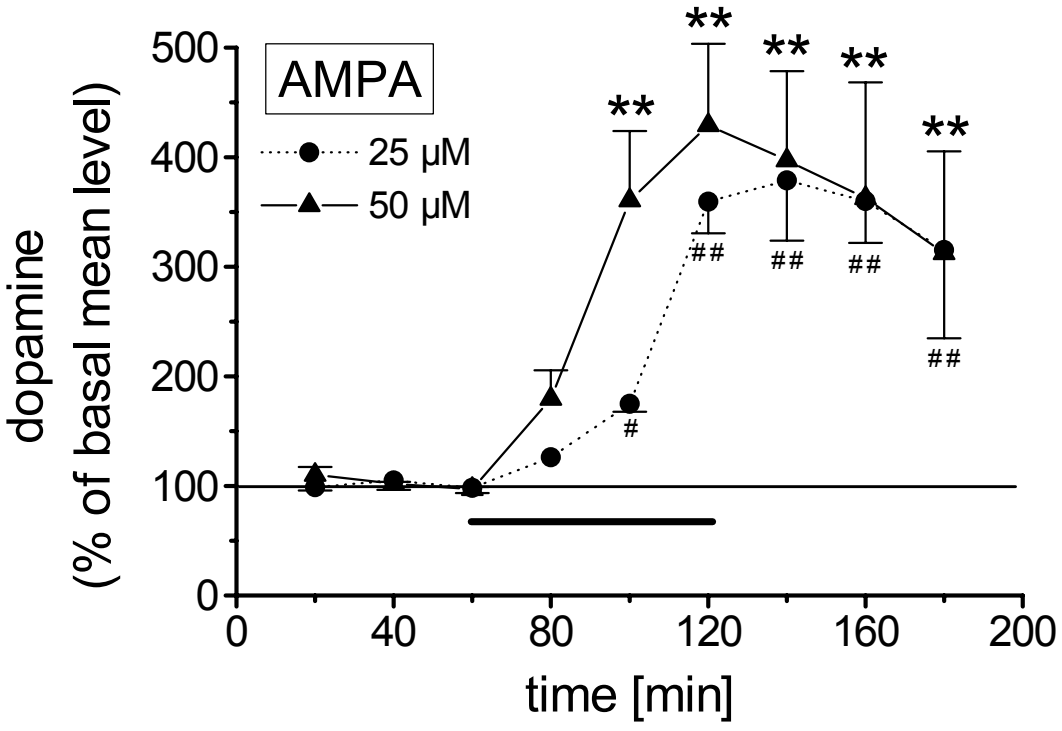

Fig. 5 Effects of AMPA infusion (25 µM, circles, n = 3 and 50 µM, triangles, n = 6) into the pedunculopontine tegmental nucleus for 1 h (indicated by bar) on the extracellular concentration of dopamine in the microdialysis samples. Data are presented as percentage of the basal mean level ± SEM. Data were analyzed by repeated measures one-way ANOVA followed by the Fischer´s LSD-(protected t) test, if appropriate. *$p<0.05$, **$p < 0.01$ significant differences for 25 AMPA data versus basal mean levels. $^{\#\#}p < 0.01$ significant differences for 50 AMPA data versus basal mean levels.

Baclofen infusion

Baclofen, 100 and 200 µM, decreased extracellular DA levels but the effect of the lower dose was more long lasting, while the higher dose had a more pronounced effect on DA levels (Table1, Fig. 6). Neither dose of Baclofen had an effect on DOPAC or HVA levels (Table 1).

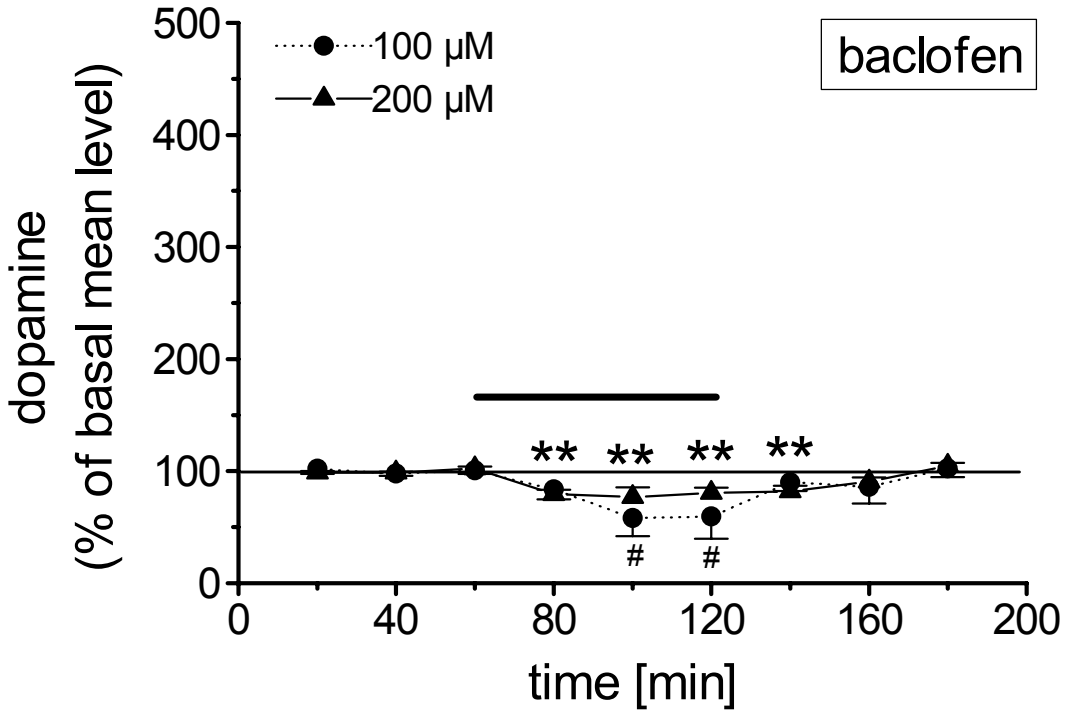

Fig. 6 Effects of baclofen infusion (100 µM, circles, n = 4 and 200 µM, triangles, n = 6) into the pedunculopontine tegmental nucleus for 1 h (indicated by bar) on the extracellular concentration of dopamine in the microdialysis samples. Data are presented as percentage of the basal mean level ± SEM. Data were analyzed by repeated measures one-way ANOVA followed by the Fischer´s LSD-(protected t) test, if appropriate. **$p < 0.01$ significant differences for 100µM baclofen data versus basal mean levels. $^{\#}p < 0.05$ significant differences for 200 µM baclofen data versus basal mean levels.

Behavior

No behavioral changes were observed after infusion of DL-amphetamine or KCl (data not presented). Likewise, neither NMDA nor AMPA receptor stimulation produced any significant behavioral changes, although five out of nine NMDA-treated animals showed moderate ipsilateral circling behavior, whereas such activation was never observed after AMPA stimulation.

Whereas the lower dose of baclofen did not produce any significant behavioral effect, the higher dose induced stereotyped behavior, which was expressed in 'activity during sitting' (Table 2). Accordingly, the 'time of inactivity' was significantly reduced (Table 2). Interestingly, this activation was only observed directly after finishing drug infusion (Table 2). Locomotor behavior was not induced, but in some cases contra-lateral circling was observed, which did not reach a significant level because of a broad variation in time.

Table 2: Effects of baclofen infusion into the pedunculopontine tegmental nucleus (PPTg) on motor activity..

Time [min] of experiment	Activity during sitting [s]	Inactive sitting [s]
62 – 80	**23 ± 25**	**1036 ±30**
82 – 100	**31 ± 21**	**1047.5 ±22**
102 – 120	**109 ± 101**	**948.75 ±134**
122 – 140	**150 ± 61***	**899.5 ±95***
142 – 160	**121 ± 48***	**905.75 ±79***
162 –180	**82 ± 40***	**918 ±104**

Baclofen (200 µM; n = 6) was infused for 1 hour 0 - 60 min into the PPTg, and subsequently infusion with aCSF was continued. The times the animals spent with active sitting (stereotyped behavior; e.g. sniffing, digging, head movements) and inactive sitting is expressed as means ± SEM for the periods indicated

*p < 0.05, **p < 0.01 compared with activity before treatment: data were analyzed using the one-sample t-test (mean compared with constants 1080 s for inactive sitting or 0 s for active sitting)

Discussion

The present results indicate that the PPTg is innervated by DA terminals and that the release of DA in the PPTg can be modulated by NMDA, AMPA and GABA. However, only the reduction of the DA signal in the PPTg by baclofen is of behavioral relevance.

The basal DA level in the PPTg was 0.077 ± 0.006 nM. In comparison with other structures of the ventral basal ganglia circuit, such as the VTA (0.3 ± 0.017 nM, Kretschmer 1999), NAC (0.949 ± 0.061 nM, Kretschmer 1999) or VP (0.58 ± 0.08 nM, Kretschmer et al. 2000), this indicates a low dopaminergic innervation of the PPTg. Neuroanatomical studies corroborate this moderate dopaminergic innervation. Thus, after injection of the retrograde marker wheat germ agglutinin-horseradish peroxidase into the PPTg Steininger et al. (1992) describes a "small to moderate number of retrogradely labeled neurons" in the SNc and "sparsely labeled regions" including the VTA. These results are in line with other neuroanatomical studies revealing a slight dopaminergic innervation of the PPTg (Semba und Fibiger 1992, Ichinohe et al. 2000). Moreover, tyrosine hydroxylase immunoreactive cells have been described by Hokfelt et al. (1984). They also indicate that cells outside the PPTg/LDTg region are free of tyrosine hydroxylase.

Nevertheless, one might speculate that the neurochemical and behavioral effects found in the present study are due to drug diffusion, since dopaminergic neurons of the VTA and SNc are anatomically close to the PPTg. Likewise, the infusion of glutamate (Westerink et al. 1996; Kretschmer 1999) and GABA ligands (Yoshida et al. 1994) into VTA or SNc produced similar neurochemical effects as found in this study. However, infusion of NMDA and AMPA into the VTA elicited strong

behavioral activation, whereas infusion into the PPTg produced only slight activation after NMDA and no activation after AMPA. Thus, we presume that the neurochemical as well as the behavioral effects reported in this study are due to a stimulation of the PPTg neurons, and are not the result of diffusion.

Neurochemistry

Stimulation of the PPTg with 100 mM KCl or 1 µM amphetamine, i.e. drugs which are considered to affect pre-synaptic neuronal activity and DA reuptake sites, respectively (Zetterstrom et al. 1986; Hurd and Ungerstedt 1988), induced a prominent DA release in the PPTg. We therefore conclude that the DA release in the PPTg is from neuronal sources.

Furthermore, we found that activation of the glutamatergic system in the PPTg stimulates, whereas activation of the GABAergic system blocks DA release in this structure. These effects are mediated by NMDA, AMPA or $GABA_B$ receptors.

This is in line with neuroanatomical and histochemical studies. It has been shown that STN and the cortex project to the PPTg probably via glutamatergic fibers (Kita and Kitai 1987; Monakow et al. 1979; Matsumura et al. 2000). In addition, GLU receptor subunits -particularly the AMPA receptor subunit GluR1, and the NMDA receptor subunit NMDAR1- are expressed in the PPTg (Watanabe et al. 1994; Inglis and Semba 1996).

In the present study, AMPA emerges as the more potent GLU receptor agonist in the PPTg since the effect on DA release is more pronounced (relative to the 20 fold higher concentration of NMDA) and more long-lasting. The reason for this temporal difference in the AMPA and NMDA receptor mediated response is unknown, but similar effects have also been reported for other structures of the ventral basal ganglia loop (NAC and VTA) by Kretschmer (Kretschmer 1999; Kretschmer et al. 2000). One interpretation might be that the two glutamate receptors may have different functional implications in terms of temporal representation of information in the basal ganglia. However, different

pharmacokinetic characteristics or receptor affinities of the two substances cannot be excluded.

The GABAergic afferents to the PPTg arise from different nuclei of the BG (Oertel and Mugnaini 1984; Steininger et al. 1992), e.g. GP (Moriizumi and Hattori 1992), VP (Semba and Fibiger 1992), SNr (Nakamura et al. 1989) and NAC (Heimer et al. 1991), but nuclei in the brainstem are also probable sources of GABAergic innervation (Childs and Gale 1984). Since studies investigating GABAergic functions in the PPTg have focused on $GABA_A$ receptors (Childs and Gale 1984; Garcia-Rill et al. 1986; Samson and Chappell 2001; Torterolo et al. 2002), but since $GABA_B$ receptors are also described in the PPTg (Chu et al. 1990), we examined the function of $GABA_B$ receptors in the PPTg. Thus, the decrease of the DA release that we found is the result of activation of $GABA_B$ receptors located in the PPTg, which are normally used by GABAergic afferents from different sources. However, neuroanatomical studies reported distinct neurons projecting from the PPTg to the mesencephalic DA neurons (Beninato and Spencer 1987; Lavoie and Parent 1994b), which contain ACh (Gould et al. 1989) and GLU (Scarnati et al. 1986) as transmitters. The topography of this projection is discussed in regard to the VTA and SNc, and some studies favor the SNc as the major target of the PPTg neurons (Blaha and Winn 1993; Oakman et al. 1995; Blaha et al. 1996). A combined innervation of VTA and SNc seems probable, since electrical and pharmacological stimulation of PPTg neurons activates both VTA and SNc (Scarnati et al. 1986; Kelland et al. 1993; Lokwan et al. 1999) and leads to a change in DA transmission in the caudate-putamen (Hernadez-Lopez et al. 1992; Chapman et al. 1997) and NAC (Niijima and Yoshida 1988; Klitenick and Kalivas 1994). This kind of cross activation has also been observed after ACh and GLU infusion (see Kalivas 1993 for review).

Furthermore, DA release in PPTg can also be the result of poly-synaptic activation. Neuroanatomical findings reveal extensive projections from the PPTg to the STN (Wolf and Butcher 1986; Mesulam et al. 1992). These neurons contain GLU and ACh (Scarnati et al. 1986; Beninato and Spencer 1987), but also GABA (Bevan

and Bolam 1995). STN itself sends glutamatergic projections to SNc (Jaffer et al. 1995). Thus, the DA release found in the present study can be induced by activation of STN neurons. Moreover, PPTg projections to VP and NAC (Semba and Fibiger 1992; Steininger et al. 1992) that innervate the VTA are described (Swanson and Cwan 1975; Pennartz et al. 1994) and can be responsible for the changes in DA release that we found after drug infusion into the PPTg. Thus, the DA release in the PPTg can be induced by three alternative circuits: (1) directly, PPTg – SNc/VTA – PPTg; (2) PPTg – STN – SNc – PPTg, and (3) PPTg – VP/NAC – VTA – PPTg.

Nevertheless, the effects on DA release that we found after NMDA, AMPA and baclofen can also be explained by the presents of pre-synaptic receptors on dopaminergic terminals within the PPTg itself. Whereas a pre-synaptic action of $GABA_B$ receptors on DA terminals has been described (Smolders et al. 1995), the existence of pre-synaptic NMDA or AMPA receptors on DA terminals is still questionable (Hattori and Fibiger 1982; Cheramy et al. 1986).

Behavior

On the basis of the neurochemical data, some modulatory effects of DA in the PPTg on behavior seem likely. Indeed, our results imply that DA release in the PPTg is negatively correlated to motor behavior. In line with our data Childs and Gale (1984) reported that contra-lateral circling occurred after the infusion of the $GABA_A$ agonist muscimol into the PPTg. In line with this observation, we saw contra-lateral circling and an increase of stereotyped behavior. Whether this behavior is, however, the exclusive result of a reduced DA release in the PPTg needs further attention, since the DA release was already reduced after 20 min while the behavioral activation was found 40 – 60 min after starting the perfusion. Furthermore, stimulated behavior was still present when the DA release had already returned to the baseline level. Changes in other regions or of other transmitters need to be considered.

Stimulation of NMDA or AMPA receptors does not affect behavior, although the ipsilateral circling observed after NMDA infusion in some animals is in line with an earlier study showing such behavior after kainate injection into the PPTg (Niijima and Yoshida 1988).

Nevertheless, we could have been expected to find more than only slight or no behavioral changes, since the PPTg is described to be a part of the 'mesencephalic locomotor region`, a region where locomotor activity can be stimulated in decerebrated rats (Shik et al. 1966; Garcia-Rill 1986). In addition, procaine infusion into the PPTg blocks locomotion induced by amphetamine infusion into the NAC (Mogenson and Wu 1988), and excitatory amino acids or radio frequency lesions of the PPTg in monkey induces bradykinesia (unilateral) or akinesia (bilateral; Kojima et al. 1997; Munro-Davis et al. 1999). Nevertheless, recent publications are contradictory regarding a role of the PPTg in mediating locomotion (Inglis et al. 1994; De Leonibus et al. 2001). Winn (1998) describes the function of the PPTg as more complex than pure locomotor controlling. He summarizes the effects found after PPTg lesion as an impairment of the stimulus reward association (see also Steckler et al. 1994; Florio et al. 1999; Inglis et al. 2000; Satorra-Marín et al. 2001), maybe via an attention deficit (Inglis et al. 2001). Accordingly, lesions of the PPTg interfere with learning (Dellu et al. 1991); reward (Bechara and van der Kooy 1989; Yeomans et al. 1993; Olmstead et al. 1998; Corrigal et al. 1999), passive and active avoidance (Fujimoto et al. 1989, 1992) and prepulse inhibition of startle, a possible measure of attention (Koch et al. 1993).

Why animals in our experiments did not show any clear behavioral responses can be explained by the experimental conditions: Animals were attached to the tether of the microdialysis setup at least 4 h before drug infusion. Thus the animals were habituated and not actively behaving at the time of drug infusion. As consequence, only general activation can be detected in this assay, but not changes of an ongoing behavior, like the performance in an operant task.

Conclusion

In conclusion, the present study demonstrates that the PPTg receives dopaminergic input, probably from the SNc and VTA (Steininger et al. 1992; Semba and Fibiger 1992, Ichinohe et al. 2000) and that this input is modulated by NMDA, AMPA and $GABA_B$ receptors. These results are in line with other studies, showing a correlation between PPTg stimulation and changed DA transmission in the caudate-putamen (Niijima and Yoshida 1988; Hernadez-Lopez et al. 1992; Blaha and Winn 1993; Chapman et al. 1997) and NAC (Niijima and Yoshida 1988; Klitenick and Kalivas 1994). Winn (1998) considers the PPTg as part of frontostriatal information processing. Our results are in favor of such a position of the PPTg that is not only downstream of the BG, but with the ability to modulate information processing via dopaminergic midbrain neurons.

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Effects of ibotenate pedunculopontine tegmental nucleus lesions on exploratory behaviour in the open field

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Abstract:

The pedunculopontine tegmental nucleus (PPTg) as part of the mesencephalic locomotor region is discussed to be involved in motor activity. In this study, we examined whether the PPTg plays a role in exploratory behaviour. Therefore, we compared non-habituated motor behaviour of PPTg lesioned rats with sham lesioned rats under spontaneous, dizocilpine (MK-801) (0.1 and 0.16mg/kg) and DL-amphetamine (1 and 2mg/kg) conditions. In order to analyse exploratory behaviour only, session-times were limited to 5 min after placing the rats in an open field. The exploratory motor activity was compared to the motor activity obtained in rats habituated to the environment.

PPTg lesions had no effect on spontaneous exploratory behaviour, but it intensified the enhanced motor activity induced by MK-801. However, PPTg lesions blocked the enhanced exploratory behaviour, i.e. horizontal activity, rearing and centre activity induced by amphetamine.

These data indicate that the PPTg is involved in behaviour driven by the dopaminergic and glutamatergic systems, when the animals are in a particular motivational state, e.g. a state that increases motor activity for itself, like exploration. This is underlined by the finding that animals exploring their environment show a higher motor activity even after multiple sessions, than animals familiar to the environment.

Keywords:

MK-801, Dizocilpine, Amphetamine, Locomotion, Motor Activity, Glutamate, Dopamine, Motivation, Mesopontine Tegmentum

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Introduction

The pedunculopontine tegmental nucleus (PPTg) receives input from basal ganglia (BG) nuclei, motor cortex, limbic areas and dopaminergic midbrain neurons [22;35;37;38] and is directly connected to other motor nuclei in the brainstem and spinal cord [9;32]. Ascending projections of the PPTg terminate in the thalamus, lateral hypothalamus, and BG [10]. This connectivity associates the PPTg in circuitries implicated in the modulations of motor functions, arousal and motivation.

In line with these anatomical data, the PPTg regulates muscle tone, postural functions during locomotion and generates and modulates neuronal rhythmic activity patterns in relation to locomotion [8;9]. Furthermore, the PPTg is part of the mesencephalic locomotor region where locomotion can be elicited in decerebrated rats [8], and it is also involved in sensorimotor gating [20]. In addition, PPTg lesions induce akinesia in monkeys [24], and rats receiving procaine injections into the PPTg, show reduced locomotion that was induced by intra-subpallidal, intra-accumbal or intra-hippocampal injections of picrotoxin, amphetamine or N-methyl-D-aspartate (NMDA), respectively [23]. Nevertheless, the implication of the PPTg in locomotion is not consistent. Other studies in rats did not show effects of PPTg manipulations on spontaneous or drug induced locomotion, i.e. locomotion induced by intra-accumbal amphetamine or dizocilpine (MK-801), intra-ventral pallidal picrotoxin, systemic amphetamine or apomorphine application following bilateral 6-hydroxydopamine lesions of the NAC [5;6;13;14;25;29;40], although, one study found an effect of PPTg lesions on amphetamine induced locomotion [4].

In view of these results an involvement of the PPTg in the modulations of motor functions is conceivable [33], but evidence demonstrates that the PPTg is not involved in locomotion per se [5;6;13;14;25;29;40]. Instead PPTg lesion studies indicate a function of the PPTg in more complex processes related to attention [16;20] or reward and motivation [3]. These deficits might be due to altered

thalamocortical activity or motor-limbic information processing, i.e. affecting attention processes or affecting response selection on striatal level, respectively [1;15;18;26;41]. For the latter dopamine glutamate interactions have a pivotal role [12;19;28;34]. And the PPTg is strongly interconnected with structures involved in striatal DA-GLU interactions [11;17;22;35;37;38;42] and is in itself a place of motor-limbic integration [36] and DA-GLU interactions [37].

We therefore studied in more detail the function of the PPTg in motor behaviour of rats exploring a new environment, i.e. in a situation of high exploratory motivation where untreated animals show motor activity. For this approach, we lesioned the PPTg with ibotenic acid and studied motor behaviour in short sessions, in saline, MK-801 (glutamate antagonist) and amphetamine (dopamine agonist) treated rats. The effects on motor activity were compared to those obtained in rats familiar with the environment.

Material and Methods

Animals

All experiments were carried out with male Sprague-Dawley rats (Charles River, Sulzfeld, Germany). Animals were housed in groups of 6 – 8 in laboratory cages (Pereg, Techniplast 95 x 44 x 21 cm) under standard conditions (temperature 22 ± 3 °C; 12 hour light/dark cycles). Food was restricted to approximately 12 g/day. Water was available ad libitum.

Two experiments were carried out: In experiment 1 (exploration) rats with PPTg or sham lesions were used, while in experiment 2 (habituation) naïve, untreated rats were used.

All experiments had been performed in compliance with the German Animal-Protection law and were approved by the local committee on animal care.

Drugs

The non-competitive NMDA receptor antagonist MK-801 hydrogen maleate (RBI, Cologne, Germany) and the functional dopamine agonist DL-amphetamine sulphate (Th. Geyer GMBH, Stuttgart, Germany) were diluted in isotonic saline solution and intraperitoneal (i.p.) injected 50 min before testing. Concentrations used were 0.1 or 0.16 mg/kg MK-801, and 1 or 2 mg/kg DL-amphetamine.

Ibotenate (Sigma-Aldrich, Taufkirchen, Germany) was made up as a 0.12 M solution in sterile 0.2 M phosphate buffer with a pH adjusted to 7.4. For preparing this concentration Ibotenate was dissolved in a minimum amount of 1 M NaOH and diluted with phosphate buffer to the final concentration.

Surgery

Rats underwent surgical and stereotaxic procedures under chloral hydrate anaesthesia (350 mg/kg i.p.). PPTg lesions were done according to studies from Rugg et al. [7;31] with slight modifications of their protocol. Shortly, bilateral PPTg lesions were carried out with at least 48 hours between each hemisphere. Ibotenate (n=12) or phosphate buffer as vehicle (n=11) were infused at two different injection sites in each hemisphere with the coordinates according to Paxinos and Watson [27], 1998: AP: -7.4 mm, ML: +- 1.8 mm, DV: -7.5 mm and AP: -8.1 mm, ML: +- 1.8 mm, DV: -7.0 mm (relative to bregma). Ibotenate or vehicle was infused in 0.02 µl steps in 10 s intervals for a total volume of 0.2 µl per injection site. The needle was then left in position for additional 300 s to allow diffusion of solution from the needle tip.

Body weights were monitored closely after surgery, and some lesioned animals needed extra feeding in single cages with sweetened wet mash or tube feeding. At least 14 days of recovery were given and food was available ad lib during that time.

Behavioural Tests

Experiment 1 (Exploration):

Exploratory motor behaviour was tested by placing the animals for 5 minutes in an open field (48 x 48 x 44 cm, TSE, Bad Homburg, Germany) that was equipped with two parallel infrared beam arrays (1 and 15 cm above the floor). Horizontal (distance travelled in meter), vertical (rearing number) and centre activity (distance travelled within the centre of the open field, i.e. 24 cm from the outer side margin) were analysed by the enclosed software (TSE). Spontaneous, drug free behaviour (no injections) was tested in sessions before and after drug treatment. Saline, MK-801 and DL-amphetamine administration was counterbalanced so that tests were performed in a within-subject design.

Experiment 2 (Habituation):

A second group of naïve, untreated animals (n = 8) were habituated to the open fields according to Olmstead and Franklin [25]. Briefly, habituation was carried out for two consecutive days (day1: 1 hour, day2: 30 min). On the third day 5 min of motor activity was analysed, starting 35 min after placing a rat in an open field.

Histology

After the last session rats were killed, their brains were fixed in formaldehyde and cut on a microtome. Sections were Nissl-stained and the localizations of the lesions were drawn onto plates taken from Paxinos and Watson [27].

Statistics

Experiment 1 (exploration):

Comparison of spontaneous exploratory data, i.e. before and after drug tests was assessed by two-way ANOVA with group (lesion or sham) as a between subject

factor and time (pre- and post drug test) as a within subject factor. ANOVA was followed by the Fischer's LSD-(protected-t)-test.
Comparison of exploratory data, i.e. drug versus saline treatment were assessed by two-way analysis of variance (ANOVA) with group (lesion or sham) as a between subject factor and dose (saline, dose 1, dose 2) as a within subject factor. ANOVA was followed by the Fischer's LSD-(protected-t)-test, if appropriate.

Experiment 1 (exploration) versus experiment 2 (habituation):

Analysis of spontaneous, post drug motor activity (exploration) versus habituated motor activity (habituation) was done by one-way ANOVA, followed by the Fischer's LSD-(protected-t)-test.

Results

Histology

Histological assessment of the lesions revealed extensive cell loss throughout the PPTg (Fig. 1). Only animals with substantial damaged PPTg (gliosis covering > 90 % of the PPTg as assessed by the observer) and relatively preserved surrounding areas (< 10 % gliosis in nuclei other than the PPTg) were included into the statistical analysis. One rat with damaged areas mainly outside of the PPTg and one rat with only partial damage of the PPTg were excluded from analysis, leaving a lesion group size of 10 animals. Nevertheless, in other rats some cell loss outside of the PPTg occurred (Fig. 1), but no nucleus despite the PPTg was completely affected by the lesions. Surrounding areas that were slightly damaged in some subjects included the cuneiform nucleus, the deep mesencephalic nucleus, the microcellular tegmental nucleus, the paralemniscal nucleus, the pontine nucleus, the retrorubral field and retrorubral nucleus and the subpeduncular tegmental nucleus. Animals with small lesions restricted to the PPTg did not differ in their

behavioural response as compared to animals with large lesions, i.e. including cell loss outside of the PPTg. Infusion tracks in control rats were always found at coordinates corresponding to the PPTg (not shown).

Details concerning the quantification of both cholinergic and non-cholinergic cell loss induced by these lesion parameters have been described elsewhere [7;14;31].

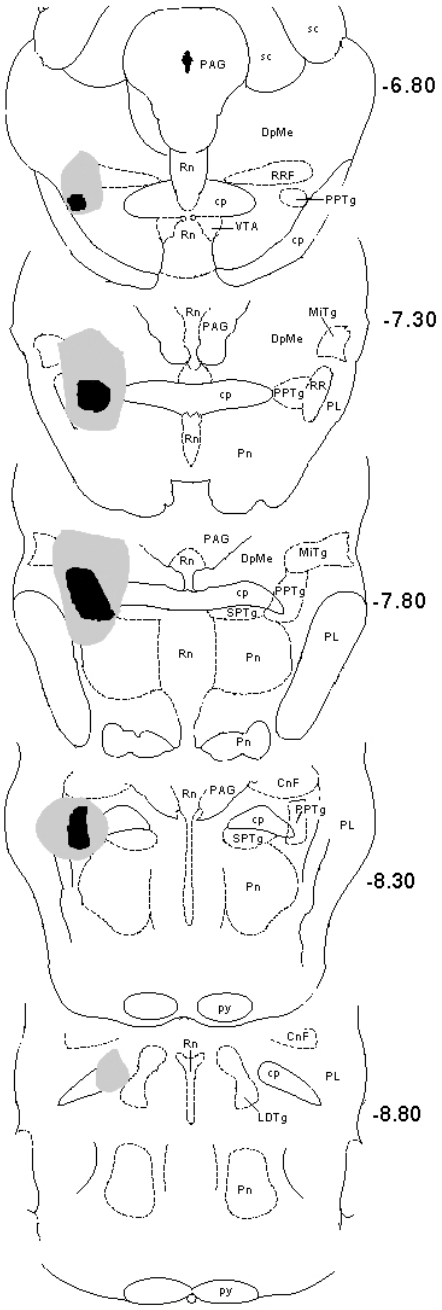

Fig. 7. Coronal sections through the rat brainstem (modified after Paxinos and Watson, 1998). Location and extent of the largest (grey areas) and smallest lesion (black area). Top section 6.8 mm and bottom 8.8 mm caudal of bregma. Abbreviations:

CnF: cuneiform nucleus, cp: cerebral peduncle, DpMe: deep mesencephalic nucleus, LDTg: laterodorsal tegmental nucleus, MiTg: microcellular tegmental nucleus, PAG: periaqueductal grey, PL: paralemniscal nucleus, Pn: pontine nuclei, PPTg: pedunculopontine tegmental nucleus, py: pyramidal tract, Rn: raphe nuclei, RR:

Spontaneous motor behaviour

Motor activity in the open field is presented in table 1. Two-way ANOVA of spontaneous exploratory motor activity revealed effects over time, i.e. horizontal activity ($F = 27.8$, $p < 0.0001$, $df = 1$), rearing ($F = 17.9$, $p = 0.004$, $df = 1$) and centre activity ($F = 4.8$, $p = 0.0397$, $df = 1$), but no main effects were found between PPTg lesioned and sham groups (horizontal activity: $F = 2.56$, rearing: $F = 0.09$ and centre activity $F = 0.76$, in all parameters $df = 1$) or time and group interaction (horizontal activity: $F = 0.26$, rearing: $F = 0.28$ and centre activity $F = 0.62$, in all parameters $df = 1$). Post-hoc analysis showed that horizontal motor activity ($t = 3.77$, $t = 3.12$), rearing behaviour ($t = 3.29$, $t = 3.22$), but not centre activity was decreased in post-drug tests compared to pre drug tests, in sham and lesioned animals, respectively.

Comparison between post-drug test exploration and habituation groups revealed a main effect for horizontal activity ($F = 11.97$, $p = 0.0002$, $df = 2$), rearing ($F = 3.60$, $p = 0.0415$, $df = 2$), but not for centre activity ($F = 2.07$, $df = 2$). Post-hoc analysis showed a higher motor activity, i.e. horizontal motor activity (sham: $t = 3.99$, lesions: $t = 4.59$) and rearing behaviour (sham: $t = 2.28$, lesion: $t = 2.46$) in non-habituated rats as compared to habituated rats.

Tab. 1: Motor activity in the open field in exploring or habituated rats.

	Exploration Pre drug tests		**Exploration** Post drug tests		**Habituation**
	Sham	Lesion	Sham	Lesion	Untreated rats
Horizontal Activity [m]	**20.9 ± 0.5**	**23.3 ± 0.9**	**14.4 ± 1.8****	**15.7 ± 1.7****	**4.7 ± 1.3**$^{€€\ \$\$}$
Rearing [#]	**38.6 ± 2.9**	**35.8 ± 3.0**	**21.3 ± 5.3****	**22.1 ± 4****	**6.8 ± 2.9**$^{€\ \$}$
Centre Activity [m]	**1.8 ± 0.3**	**1.8 ± 0.3**	**1.4 ± 0.6**	**0.8 ± 0.2**	**0.2 ± 0.1**

Columns 1 and 2 present motor activity during exploration of PPTg and sham lesioned rats before and after drug treatment, respectively. Column three presents motor activity of habituated rats. Analysed parameters were: A. horizontal activity [m], B. rearing behaviour [#] and C. centre activity [m]. $^{*}P < 0.05$, $^{**}P < 0.01$ significantly different to pre drug test. $^{€}$ $P < 0.05$, $^{€€}$ $P < 0.01$ significantly different to sham treated exploration group. $^{\$\$}$ $P < 0.01$ significantly different to PPTg lesioned exploration group. Data presented as mean ± S.E.M. For t-values see text.

Amphetamine

The effects of PPTg lesions on exploratory motor behaviour after amphetamine administration are shown in Fig. 2. ANOVA revealed main effects of dose (horizontal activity: $F = 5.93$, $p=0.004$; rearing: $F = 4.22$, $p = 0.0183$; centre activity: $F = 7.86$, $p = 0.0008$; in all cases $df = 2$) in all parameters. Main effects on group was not consistent, there was no effect in horizontal activity ($F = 2.24$, $p=0.138$, $df = 1$), but in rearing ($F = 4.62$, $p = 0.0348$, $df = 1$) and centre activity ($F = 7.91$, $p = 0.0062$, $df = 1$). Dose by group interaction were found in all parameters (horizontal activity: $F = 7.96$, $p=0.0007$; rearing: $F = 8.43$, $p = 0.0005$; centre activity: $F = 9.36$, $p = 0.0002$; in all parameters $df = 2$).

Post–hoc analysis showed that PPTg lesions blocked the enhanced motor behaviour induced by the higher concentration of DL-amphetamine (sham versus lesion:

horizontal activity: t = -3.48; rearing: t = 3.87; centre activity t = 4.36), which was expressed in sham lesioned animals by an increased horizontal motor activity (t = -4.74), rearing behaviour (t = -4.55) and centre activity (t = -4.69) as compare4d to saline treated animals. The lower concentration had no significant effect on the behaviour in both groups (Fig. 2 A-C).

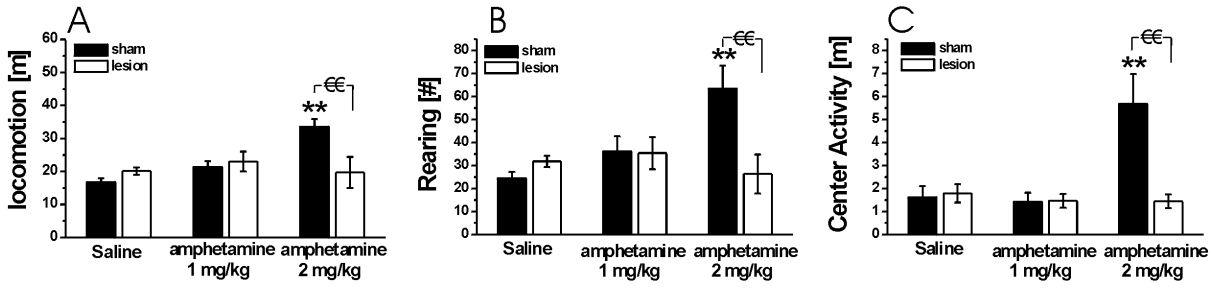

Fig. 2. Bar diagram illustrating the effects of PPTg lesions on exploratory behaviour after DL-amphetamine challenge (1 and 2 mg/kg, i.p.) in the open field. Analysed parameters were: A. horizontal activity [m], B. rearing behaviour [#] and C. centre activity [m]. **P < 0.01 significantly different from saline/corresponding group (sham or lesion); €€ P < 0.01 significantly different from sham/same treatment. Data presented as mean ± S.E.M. For t-test values see text.

MK-801

The effects of PPTg lesions on exploratory motor behaviour after MK-801 administration are shown in Fig. 2. ANOVA revealed main effects of dose (F = 33.36, p < 0.0001, df = 2), group (F = 5.97, p = 0.0168, df = 1) and dose x group interactions (F = 4.18, p = 0.0189, df = 2) for horizontal activity, but no main effects were found for rearing or centre activity.

PPTg lesions acted synergistic with MK-801 on behaviour, which was characterized by an increased horizontal motor activity (sham: t = -3.25, t = -6.36; lesions: t = -5.47, t = -4.55 for 0.1 and 0.16 mg/kg MK-801 versus saline, respectively). Thus, the lower concentration of MK-801 had a stronger impact on

horizontal activity in lesioned animals (t = -2.99, Fig3A) than in sham treated animals. Nevertheless, both concentrations of MK-801 had no effect on rearing behaviour or centre activity (Fig. 3B and 3C).

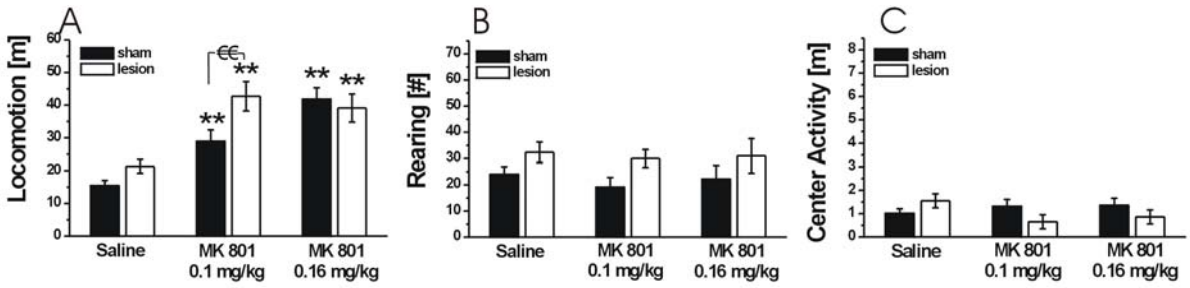

Fig. 3. Bar diagram illustrating the effects of PPTg lesions on exploratory behaviour after MK-801 challenge (0.1 and 16 mg/kg, i.p.) in the open field. Analysed parameters were: A. horizontal activity [m], B. rearing behaviour [#] and C. centre activity [m]. **P < 0.01 significantly different from saline/corresponding group (sham or lesion). €€ P < 0.01 significantly different from sham/same treatment. Data presented as mean ± S.E.M. For t-test values see text.

Discussion

Spontaneous motor behaviour

The present study demonstrates that ibotenate lesions of the PPTg did not change spontaneous horizontal motor activity, rearing and centre activity in the open field during the exploratory phase. This result is in line with other studies [5;6;13;14;25;29;40].

One important issue for the interpretation of our results is that tests were carried out during the exploratory phase of the animals that is characterized by an increased motor activity compared to habituated rats (see Table 1). Even though exploratory

motor behaviour decreased over the experimental days (Table 1 columns 1 and 2), exploration of an environment, for the first time or after multiple sessions on different days, still generates an increased motor activity compared to habituated animals (Table 1: comparison of columns 1 and 2 with column 3). This increase is the result of a persisting motor activation driven by exploratory motivation in all sessions. Interestingly, the PPTg seems to have no implications in this behaviour.

Amphetamine

PPTg lesions blocked the amphetamine induced increase in horizontal motor activity, rearing and centre activity during exploration. This prominent result is surprising, since other authors found no effect of PPTg lesions on horizontal motor activity induced by systemic amphetamine [13;25]. An explanation for the blockade found in our study might be the differences in the protocols. In our exploration experiment no habituation sessions were performed prior to amphetamine sessions. In addition, animals stayed for only 5 minutes during the peak time of drug effects in the open fields. Thus, the analysed behaviour was driven by a combination of a drug effect and exploratory motivation. In contrast, animals in the studies reporting no effect of PPTg lesions on amphetamine induced behaviour, were already habituated to their environment for a long period (Inglis et al.: habituation sessions: 1h/day for 21 days, drug session: 1h before drug administration [13] and Olmstead et al.: habituation sessions: 30 and 60 min/day for 2 days, respectively, drug session: 1h before drug administration [25], before drug effects on motor behaviour was tested. Thus, these animals were in view of motivational aspects in a different situation than rats in our study. In line with our results, one other study reported that PPTg lesions block locomotor excitation produced by systemic amphetamine [4]. Animals in this study were left in the open field for 6 min, therefore recording exploratory behaviour and supporting our hypothesis. But injections in this study were made during the 6 min session. The interpretation of these results are difficult; since motor behaviour was not measured

during the peak time of drug effects and because stress due to the injections might have affected motor behaviour.

Another explanation for the blockade of amphetamine induced increase in horizontal motor activity by PPTg lesions might be an increase of stereotypies in lesioned animals. In this case lesioned animals would react more pronounced on amphetamine displaying stereotypies rather than horizontal activity. In fact Inglis and colleagues [13] reported increased levels of stereotypies like biting, licking and gnawing in PPTg but not sham lesioned rats after 3 and 5, but not 1.5 mg/kg amphetamine (i.p.) administration. Since we have not analysed stereotyped behaviour in the present study we cannot exclude this interpretation of our data.

Nevertheless, we want to emphasise on several issues: First, Inglis et al. [13] reported levels of self-mutilation, severe self-licking or gnawing after 3 and 5 mg/kg amphetamine that were high enough to prevented testing for horizontal activity in open fields. These behaviours, however, were never observed in our study. Second, the doses of amphetamine after which increased levels of stereotypies were reporter are considerably higher than the high dose of amphetamine used in the present study. 2 mg/kg amphetamine might still be below the level to induce stereotypies in PPTg lesioned rats. And third, a more recent study did not find increased stereotypies even after a sensitisation protocol of seven consecutive amphetamine administrations [2]. Thus, the relationship between PPTg lesions and amphetamine-induced stereotypies is not straightforward.

Recapitulatory, our results clearly show that the PPTg is involved in behaviour driven by the dopaminergic system in a situation of motivated exploratory behaviour. This is further underlined by our experiments, showing that animals exploring the environment show a higher motor activity even after multiple sessions, than animals familiar to the environment (Table 1).

MK-801

PPTg lesions had a synergistic effect with MK-801 on horizontal motor activity. To our knowledge this is the first study which links the PPTg to motor activity induced

by systemic glutamate antagonism. Thus, it is not known whether PPTg lesions affect Mk-801 induced motor activity only in a situation of motivated exploratory behaviour, as it is the case in amphetamine induced motor activity, or whether it is a more general effect. Nevertheless, one study in habituated rats showed that locomotion induced by local infusion of MK-801 into the NAC is not affected [5] by PPTg lesions. Several explanations for the differences to our finding might be considered: First, the effect in our study can be due to actions of MK-801 outside of the NAC. Second, we found augmented motor effects in lesioned rats only after the lower dose, but not after the higher dose of MK-801. This could be due to a ceiling effect, which could also explain the missing effect of accumbal MK-801 [5]. And third, a similar mechanism as in the amphetamine study might be argued. Thus, PPTg lesions might only affect MK-801 induced behaviour in a situation of motivated exploratory behaviour. Even though, it is well known that MK-801 and amphetamine induce stereotyped behaviour, several differences exist between the behavioural effects of the two drugs. Interestingly, amphetamine and MK-801 induced stereotypies with different qualities as seen in sham-lesioned rats. Amphetamine increased horizontal motor activity, rearing behaviour and centre activity, while the effect of MK-801 is restricted to horizontal motor activity. Rückert et al. [30] demonstrated that behaviour induced by systemic MK-801 is not guided by environmental stimuli. Thus, MK-801 enhances stereotyped running rather than stereotypies of exploratory behaviours [30]. In addition, PPTg lesions block the complete behavioural motor pattern (horizontal motor activity, rearing behaviour and centre activity) of amphetamine, and in contrast potentiate that of MK-801 (horizontal motor activity). These data suggest that the motor stimulating effect of the NMDA receptor antagonist MK-801 and of the dopamine agonist amphetamine on motor activity in the open field is mediated in a different way. A concept that is consistent with other studies [20]. However, it remains speculative whether PPTg lesions affect behaviours driven by glutamate antagonism in general or only situation dependent.

Conclusion

It is shown that the PPTg is not involved in motor behaviour per se, i.e. PPTg lesions do not interfere with the ability to process spontaneous or drug induced motor behaviour. Nevertheless, PPTg lesions do interfere with motor behaviours driven by the dopaminergic or glutamatergic systems when the animals are in a particular motivational state, e.g. a state that increases motor activity for itself, like exploration. Interestingly, a dependency of PPTg lesion effects on the state of the animal was also observed for behaviours other than locomotion: Conditioned place preference (CPP) to food, opiates or amphetamine is only blocked in satiated and drug-naïve rats, but not in food-deprived or drug-dependent rats [3;4;25]. Thus, also the modulation of CPP by the PPTg depends on the motivational state of the animals. Furthermore, the PPTg is known to participate in processes involving sleep-wake cycle and arousal [39], i.e. processes defining the vigilance and influencing the motivational state of animals [8;36]. In the scope of these data, the PPTg, cholinergic and non-cholinergic neurons, might be described as a possible link between arousal state and the motor system [21]. Our data indicate that the modulation of motor behaviour by the PPTg depends on the situation or state of the subjects.

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III

III. Steiniger-Brach & Kretschmer Psychopharmacology

(eingereicht)

Performance of pedunculopontine tegmental nucleus lesioned rats in an operant discrimination task

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Abstract

Dopamine/glutamate interactions on striatal/nucleus accumbens level are involved in the control of response selection processes, influencing motor, but also cognitive behaviors. The pedunculopontine tegmental nucleus, part of the mesopontine tegmentum is strongly interconnected with structures involved these interactions. Therefore it might be speculated that the pedunculopontine tegmental nucleus shares functional properties with this system.

Therefore we investigated interactions between the PPTg, the dopamine and glutamate systems on complex behavior, using the operant discrimination task. In this task rats were trained in an operant chamber to discriminate between an unrewarded and a fixed ratio 10 rewarded lever. Thereafter ibotenic acid or phosphate buffer was used for bilateral PPTg or sham lesions, respectively. The performance in the operant discrimination task of PPTg or sham lesioned rats treated with dizocilpine (MK-801), DL-amphetamine or saline was compared.

Lesion itself produced a dramatic decrease in response accuracy and response rate. MK-801 treatment showed impairment of accuracy as well as responding in sham-lesioned and more prominent in PPTg lesioned animals. DL-amphetamine treatment was without an effect in sham lesioned animals but impaired accuracy but not responding in lesioned animals.

These data show that the performance in the operant discrimination task is PPTg depending. Furthermore, interactions of the PPTg with the glutamatergic as well as the dopaminergic system seem to be involved in the correct processing of the operant discrimination task. Therefore, the PPTg depending behavioral alterations might involve response selection processes on striatal/nucleus accumbens level.

Introduction

Dopaminergic midbrain neurons in the substantia nigra (SN) and ventral tegmental area (VTA) respond to a wide category of salient stimuli, e.g. reward, novelty and aversion (Horvitz 2002; Salamone & Correa 2002). Furthermore, this mesoaccumbal but also the nigrostriatal dopamine (DA) system are involved in behavioral control, e.g. motor and cognitive behaviors (Fiorillo, Tobler *et al.* 2003; Salamone, Correa *et al.* 2003; Salamone 2002; Robbins, Roberts *et al.* 1983). Even though the precise role of the DA system in this process is not clear, DA is thought to be more responsible for response selection, i.e. a process that enables to select the response choice, by facilitating appropriate and inhibiting inappropriate motor responses (Koch, Schmid *et al.* 2000; Schmidt 1998; Salamone, Cousins *et al.* 1997). The most likely mechanisms through which DA influences this process is via the interaction with glutamate (GLU). More precisely, DA in the dorsal and ventral striatum (STR/NAC) modulates processing of concurrent GLU from cortex, thalamus and limbic structures which is carrying sensory, motor and motivational information (Horvitz 2002; Schmidt 1998; Pennartz, Groenewegen *et al.* 1994).

The pedunculopontine tegmental nucleus (PPTg) is strongly interconnected with structures involved in DA-GLU interactions on STR/NAC level. It receives dopaminergic input from SN and ventral tegmental area (VTA), glutamatergic input from motor cortical and limbic areas and it receives direct and indirect input from the STR/NAC, respectively (Steiniger & Kretschmer 2003; Semba & Fibiger 1992; Steininger, Rye *et al.* 1992; Heimer, Zahm *et al.* 1991). Furthermore, it sends ascending projections containing GLU and acetylcholine (ACh) to dopaminergic midbrain neurons, glutamatergic limbic areas, like the amygdala, and to STR/NAC and their output structures including thalamic nuclei (Oakman, Faris *et al.* 1995; Hallanger & Wainer 1988; Jackson & Crossman 1983). This connectivity pattern incorporates the PPTg in a circuit that modulates functioning of DA-GLU interactions in STR/NAC, and therefore connects the PPTg to motor and cognitive behaviors (Winn 1998; Steckler, Inglis *et al.* 1994).

More precisely, lesion studies support a role of the PPTg in the modulation of complex behavior. Responding to lateral hypothalamus intracranial self-stimulation or intravenous heroin administration (Olmstead, Munn *et al.* 1998; Lepore & Franklin 1996), as well as development of a conditioned place preference to several drugs of abuse or natural reinforcers are inhibited by PPTg lesions (Kippin & van der 2003; Bechara, Nader *et al.* 1998; Olmstead & Franklin 1997; Bechara & van-der-Kooy 1989). Furthermore, Inglis and colleagues showed that PPTg lesioned rats are able to acquire responding to a conditioned reinforcer, however, without the ability to discriminate between rewarded and non-rewarded levers (Inglis, Olmstead *et al.* 2000; Inglis, Dunbar *et al.* 1994). These results implicate that the PPTg is linked to reward related processes. However, investigations examining consumption, choice or contrast effects of different sucrose solutions indicate that PPTg lesions do not disrupt primary motivation or the ability to evaluate and respond to changes in reward strength, but block the appropriate response choice (Alderson, Jenkins *et al.* 2001; Olmstead, Inglis *et al.* 1999). It is speculated that this results from a deficit in processing of limbic information, related to response selection mechanisms (Keating & Winn 2002;Inglis, Dunbar *et al.* 1994; Alderson, Jenkins *et al.* 2001; Winn 1998). Alternatively, it can be speculated that attention deficits, caused by altered thalamocortical projections, might be the underlying mechanism for the above reported effects (Alderson, Brown *et al.* 2002; Inglis, Olmstead *et al.* 2001; Inglis, Olmstead *et al.* 2000; Olmstead, Inglis *et al.* 1999).

In view of these behavioral findings and the close relationship between the PPTg and structures involved in DA-GLU interactions on STR/NAC level, a function of the PPTg in response-selection-mechanisms is conceivable. Nevertheless, no data exist that evaluate the precise role the PPTg exerts on behavior by modulating DA-GLU interactions. For this reason we investigated the function and the interaction of the PPTg with the dopaminergic and the glutamatergic system using the conditioned operant discrimination task (ODT). Therefore, we treated PPTg- or sham lesioned rats with the non-competitive NMDA receptor antagonist dizocilpine

(MK-801), the DA agonist DL-amphetamine or saline and compared their performance in the ODT.

Materials and Methods

Animals

All experiments were carried out with male Sprague Dawley rats (Charles River, Sulzfeld, Germany). Animals were housed in groups of six in laboratory cages (Pereg; Techniplast 95 x 44 x 21 cm) under standard conditions (temperature 22 ± 3 °C; light from 6 a.m. to 6 p.m.). Food was restricted to approximately 12 g/day, including pellet-intake during behavioral training and testing. If necessary animals were fed 1 to 2 h after behavioral testing. Body weights were monitored regularly. Tap water was available ad libitum.

Experiments were done in accordance with the ethical guidelines regarding the care and use of animals and were approved by the local council of animal care (ZP1/00).

Drugs

MK-801 hydrogen maleate (RBI, Cologne, Germany) and DL-amphetamine sulphate (Th. Geyer GMBH, Stuttgart, Germany) were diluted in isotonic saline solution and intraperitoneally (i.p.) injected 30 min before testing. Doses used were 0.1 and 0.16 mg/kg MK-801, and 1 and 2 mg/kg DL-amphetamine.

Ibotenate (Sigma-Aldrich, Taufkirchen, Germany) was prepared as a 0.12 M solution in sterile 0.2 M phosphate buffer with a pH adjusted to 7.4. For preparing this concentration ibotenate was dissolved in a minimum amount of NaOH and diluted with phosphate buffer to the final concentration.

Operant chamber apparatus

Four operant chambers (24 x 21 x 30 cm) (Med Associates, St. Albans, UK) were used. Each chamber was supplied with two retractable levers, a food dispenser with an external pellet cup and two stimulus lights above each retractable lever. The experiments were controlled online by a computer system (SmartControl-Interfaces, MedPC-Software, Med Associates).

Open field apparatus

Two open fields (48 x 48 x 44 cm) (TSE, Bad Homburg, Germany) were used to measure locomotor activity. Each open field was equipped with two parallel horizontal infrared beam arrays, 1 and 15 cm above the floor to record horizontal (locomotion, expressed as distance moved [m]) and vertical (rearing, expressed as number of events [#]) activity, respectively. Beam interrupts were registered online by the enclosed software program (TSE).

Operant procedures

Before surgery, animals were trained over a period of 50 days in the ODT. Training and testing in the ODT was performed during day light approximately at the same time each day. All animals were trained once daily. On the first training day, a lever and a stimulus-light on one side of the chamber was presented. The side of the presentation was randomly chosen, but equally distributed over the whole population of rats. The lever was rewarded with a fixed ratio (FR) 1, i.e. one lever press resulted in the delivery of one 45 mg food pellet (Formula P, P.J. Noyes Company, Inc., Lancaster, USA). The session terminated after the delivery of 100 pellets. On the second training day, the reward/light associated lever was presented on the opposite side as on training day 1. Additionally, an unrewarded lever was presented. Pressing this unrewarded lever resulted in a false count but had no further consequences. On the third day, the sides of the rewarded and unrewarded levers presentation were randomly selected. Additionally, an interval protocol was

introduced. Therefore, levers were rejected 10 min after their presentation and the stimulus light was turned off. After 10 s levers and light reappeared. The side of the reward/light associated levers was again randomly selected. The training finished after 20 min. On the following 13 training days, the FR criterion was gradually increased to the final FR 10 and the lever presentation durations were decreased to random periods between 1 – 2 min. This training continued until criteria of 95 % correct responses (accuracy) and a minimum of 80 lever presses/min (response rate) were achieved for five consecutive days. Thereafter, rats were divided into two groups (ibotenate or sham lesioned) with similar overall average of accuracy and response rate.

Surgery

In order to maximize recovery, bilateral lesions were carried out with at least 48 hours between each hemisphere according to Inglis, Dunbar *et al.* 1994.
Animals were anaesthetized with chloral hydrate (350 mg / kg i.p.) and placed in a stereotaxic frame with the incisor bar positioned at approximately –3.3 mm (Paxinos & Watson 1998). Lesions of the PPTg were done with slight modifications according to studies from Rugg and Dunbar (Dunbar, Hitchcock *et al.* 1992; Rugg, Dunbar *et al.* 1992). Infusion of ibotenate (N = 13) or vehicle (N = 10) were done at two different injection sides in each hemisphere (coordinates according to (Paxinos & Watson 1998): -7.4 mm posterior, -1.8 mm lateral, –7.5 mm ventral, and -8.1 mm posterior, -1.8 mm lateral, -7.0 mm ventral to Bregma). 1 µl syringes, attached to the stereotaxic frame, were used to infuse 0.2 µl ibotenate or vehicle in 0.02 µl steps with 10 s intervals per injection. The needle was then left in position for additional 300 s to allow diffusion of solution from the needle tip. Body weights were monitored closely after surgery. Some lesioned animals needed extra feeding in single cages with sweetened wet mash or tube feeding. At least 21 days of recovery were given before testing started, during that time food was available ad libitum for lesioned rats.

Behavioral testing

Rats were tested in the ODT under spontaneous conditions, i.e. drug free behavior (no injections), for three consecutive days. Thereafter, a period of drug testing followed. Saline, MK 801 and amphetamine administration was counterbalanced so that drug tests were performed in a within-subject design. Spontaneous behavior was also tested after drug tests for three days, but with an interval of 21 days between spontaneous test days 5 and 6. Additionally, motor behavior was tested in an open field for 5 min consecutive to the first spontaneous test after surgery.

Histology

After the last session rats were killed, their brains were fixed in formaldehyde and cut on a microtome. Sections were Nissl-stained and the localizations of the lesions were drawn onto plates taken from Paxinos & Watson 1998.

Statistics

Open field activity data were compared using the Student's t-test.
Analysis of spontaneous data on accuracy and response rate of ODT performance was done using a repeated measures two-way analysis of variance (ANOVA) with groups (sham or lesion) as a between subject factor and time as a within subject factor. Comparisons of drug effects on the accuracy and response rate of ODT performance were assessed by one-way ANOVA for lesion and for sham treated animals, respectively. All ANOVA's were followed by the Fischer's LSD-(protected-t)-test if appropriate. The level of significance was set at $p < 0.05$. Data are presented as means ± S.E.M..

Results

Histology

Histological assessment of the lesions revealed extensive cell loss throughout the PPTg. Only animals with substantial damaged PPTg and relatively preserved surrounding areas, i.e. clearly < 10 % gliosis in nuclei other than the PPTg, assessed on the slide with the largest extensions, were included into the statistical analysis (Fig. 1). Four rats in total which did not meet these criteria were excluded from analysis. In three out of these four rats great damage was found outside of the PPTg. The damaged areas included the deep mesencephalic nucleus, the retrorubral nucleus, the microcellular tegmental nucleus and the cuneiform nucleus. The remaining one rat had only partial damage in the PPTg and was also excluded from analysis, even though its behavior did not differ from lesioned rats which were included in the analysis. Rats included into the analysis showed only minor cell loss outside of the PPTg (Fig. 1). No nucleus despite the PPTg was substantially affected by the lesions. Surrounding areas that were slightly damaged in some animals included the pontine nucleus, the paralemniscal nucleus, the retrorubral field and retrorubral nucleus, the deep mesencephalic nucleus, the microcellular tegmental nucleus and the parabrachial nucleus. Infusion tracks in control rats were always found at coordinates corresponding to the PPTg (not shown).

Details concerning cholinergic and non-cholinergic cell loss induced by ibotenate lesions have been described elsewhere (Inglis, Dunbar *et al.* 1994; Dunbar, Hitchcock *et al.* 1992; Rugg, Dunbar *et al.* 1992).

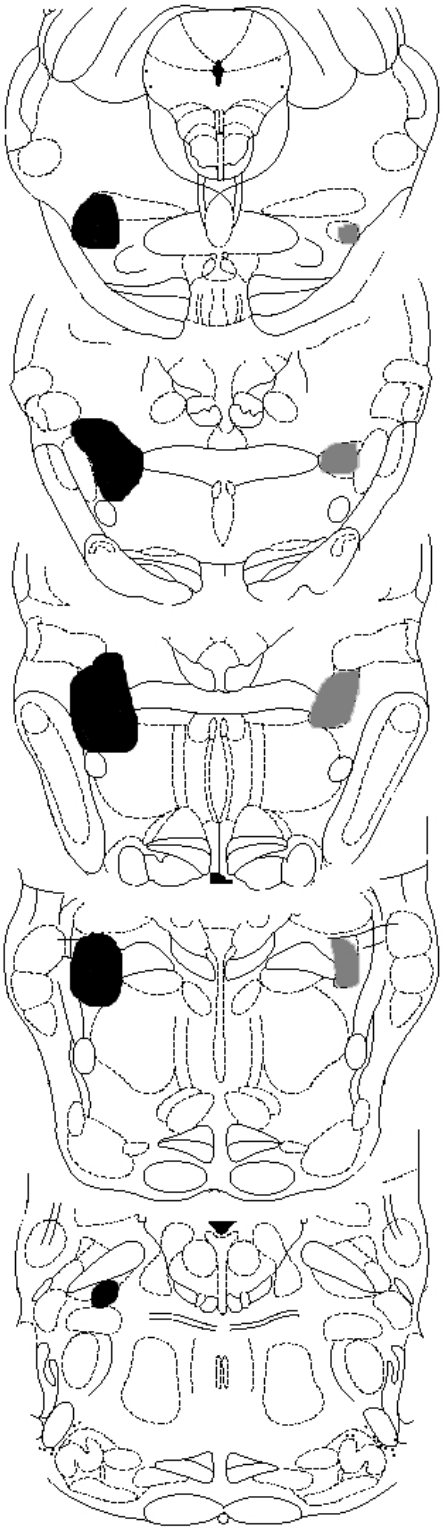

Fig. 8. Coronal sections through the rat brainstem (modified after (Paxinos and Watson, 1998)). Location and extent of the largest (grey areas) and smallest lesion (black area). Top section 6.8 mm and bottom 8.8 mm caudal of bregma. showing the extent of PPTg lesions (n = 9).

Spontaneous behavior

Open field

As shown in Figure 2 no differences were observed between PPTg lesioned and sham lesioned animals in spontaneous motor activity in the open field, i.e. locomotion (Student's *t*-test: $p > 0.1$) or rearing behavior (Student's *t*-test: $p > 0.7$) (Fig. 2).

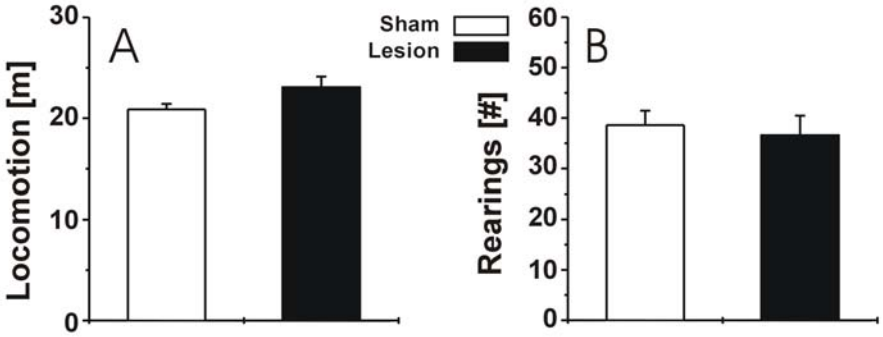

Fig. 9. Motor activity measured in the open field. Analyzed parameters were: (A) locomotion [m], (B) rearing behavior [#]. Student's *t*-test analysis of spontaneous motor behavior revealed no effect between PPTg lesioned and sham groups, i.e. locomotion or rearing behavior. Data presented as mean ± S.E.M.

Operant chamber – Operant discrimination task (ODT)

ODT performance under drug free conditions is presented in Fig. 3. Two-way ANOVA assessing the response accuracy and the response rate in the ODT, respectively, revealed main effects over time: accuracy ($F=5.9$, $p < 0.0001$) and response rate ($F=15.3$, $p < 0.0001$), as well as between groups: accuracy ($F=75$, $p < 0.0001$) and response rate ($F=28.2$, $p < 0.0001$).

Excitotoxic PPTg Lesion effects

Post hoc analysis revealed that PPTg lesions attenuated the accuracy as well as the response rate in the ODT (see below):

Accuracy: As illustrated in Fig. 3A, response accuracy of PPTg lesioned animals was significantly lower after surgery (first spontaneous test 'day 1') than before surgery (last training 'day -1'), i.e. 77.1 ± 3.6 % as compared to 94.4 ± 1.6% correct responses, respectively. Even though a slight recovery was found on spontaneous test 'day 2' (86.4 ± 1.3 %), the attenuation of the accuracy was long lasting and persisted throughout the experiment, i.e. after drug sessions and after a break of 21 days without testing (spontaneous test 'day 6': 85.7 ± 5.1 %). In sham lesioned animals, surgery had no effects on the task accuracy (Fig 3A), i.e. 95.6 ± 0.9 % (last training 'day -1') as compared to 93.7 ± 1.4 % (first spontaneous test 'day 1').

Response rate: The response rate of PPTg and sham lesioned animals was lower on spontaneous test 'day 1' (26.2 ± 4.5 and 62.7 ± 6.7 responses/min, respectively) than before surgery (training 'day -1': 87.9 ± 10.6 and 88.9 ± 9.3 responses/min, respectively) (Fig. 3B). Comparison between PPTg and sham lesioned groups revealed a stronger surgery effect on PPTg than on sham lesioned animals, this difference was long lasting and maintained to the end of the experiment, i.e. after drug sessions and a break of 21 days without testing (spontaneous test 'day 6': 50.2 ± 7.5 and 85.5 ± 6.9 responses/min, respectively). Within group comparisons revealed a recovery from surgery in sham lesioned animals, already at the 2nd spontaneous test day ('day 2') (74.5 ± 7 responses/min). No significant recovery of the response rate was found in PPTg lesioned animals at any time of the experiment.

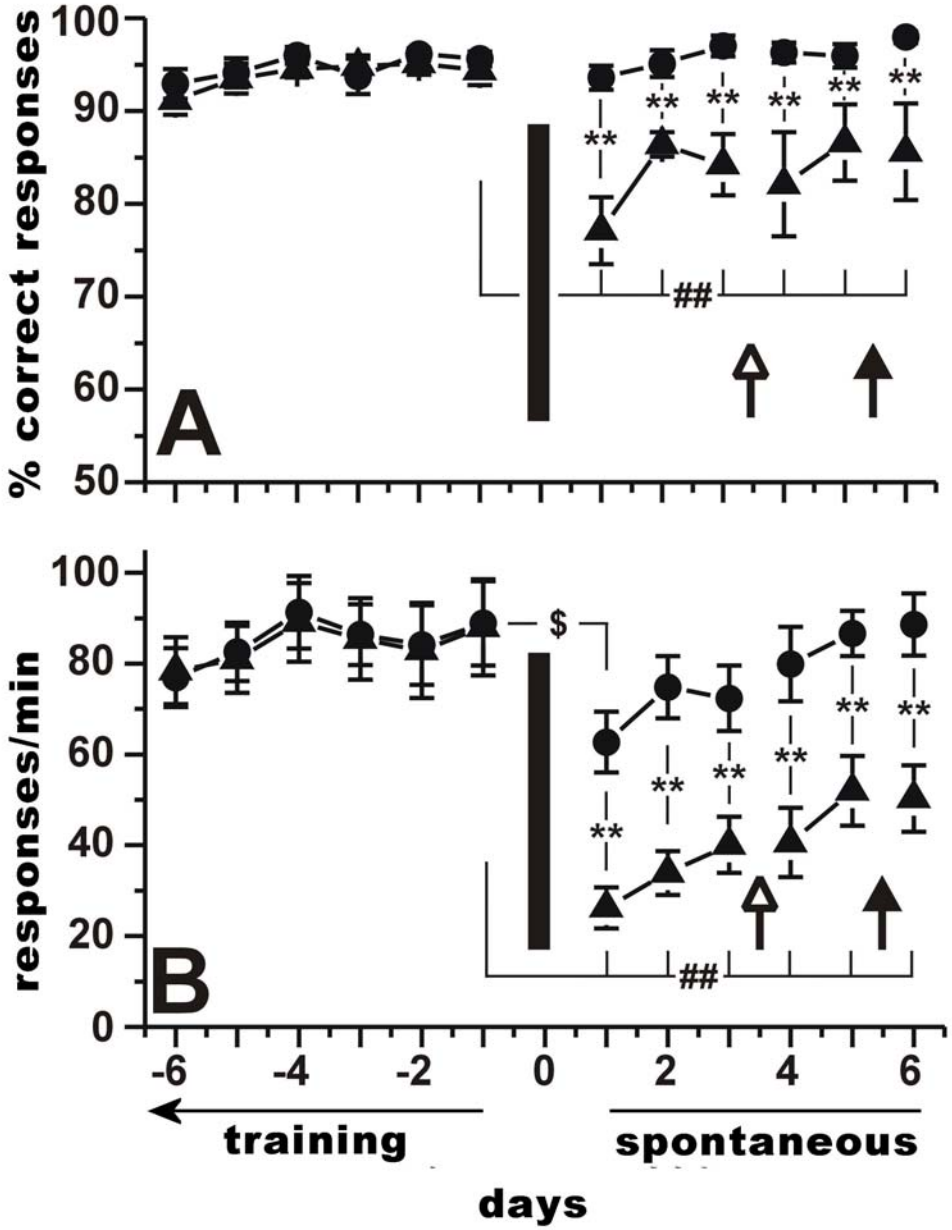

Fig. 10. Performance in the operant discrimination task: before (training) and after (spontaneous 'drug free') PPTg/sham lesions. (A) Task accuracy expressed as % correct responses. (B) Response rate expressed as spontaneous responses/minute. Vertical black bars represent the time of surgery and recovery. Open arrows indicate the period of drug application tests (see Fig. 4). Solid arrows indicate a period of 21 days without testing. Post hoc analysis was done by Fischer's LSD-(protected-t)-test, if appropriate. **P < 0.01 significantly different from sham lesioned group on the corresponding test day. ## p < 0.01 significantly different to PPTg lesioned group on the last test day before surgery (spontaneous test day -1). $ p < 0.05 significantly different to sham lesioned group on test day -1. Data presented as mean ± S.E.M.

Spontaneous performance before and after drug administration

Note that comparisons of spontaneous ODT performance on test days before and after drug administration (spontaneous tests 'day 4' and 'day 5' as compared to test 'day 3') revealed no changes in accuracy or response rate, of sham or PPTg lesioned animals (Fig.3).

Drug induced operant behavior

The effects of drug treatment (MK-801 and DL-amphetamine) on the performance of sham or PPTg lesioned animals in the ODT are shown in figure 4. ANOVA's revealed main effects of drug treatments on the ODT performance, i.e. accuracy (sham: $F = 8.05$, $p < 0.0001$ and lesion: $F = 6.83$, $p < 0.0001$) (Fig. 4A and B) and response rate (sham: $F = 6.17$, $p = 0.0003$ and lesion: $F = 5.96$, $p = 0.0004$) (Fig. 4C and D), for sham and ibotenate lesioned animals, respectively.

Post-hoc analysis of sham lesioned animal data revealed an attenuated response accuracy ($p < 0.01$) (Fig. 4A) as well as response rate ($p < 0.01$) (Fig. 4B) after 0.16 mg/kg MK-801 compared to saline administration. No other significant changes compared to saline treatment were observed in sham lesioned animals.

Post-hoc analysis of ibotenate lesioned animal data revealed that response accuracy (Fig. 4B) and response rate (Fig. 4D) were attenuated after 0.1 ($p < 0.01$, respectively) and 0.16 mg/kg ($p < 0.01$, respectively) MK-801 as compared to saline-treated ibotenate lesioned animals. Furthermore, an attenuated accuracy, but not response rate was found in ibotenate lesioned animals after 1 ($p < 0.05$) and 2 ($p< 0.05$) mg/kg DL-amphetamine.

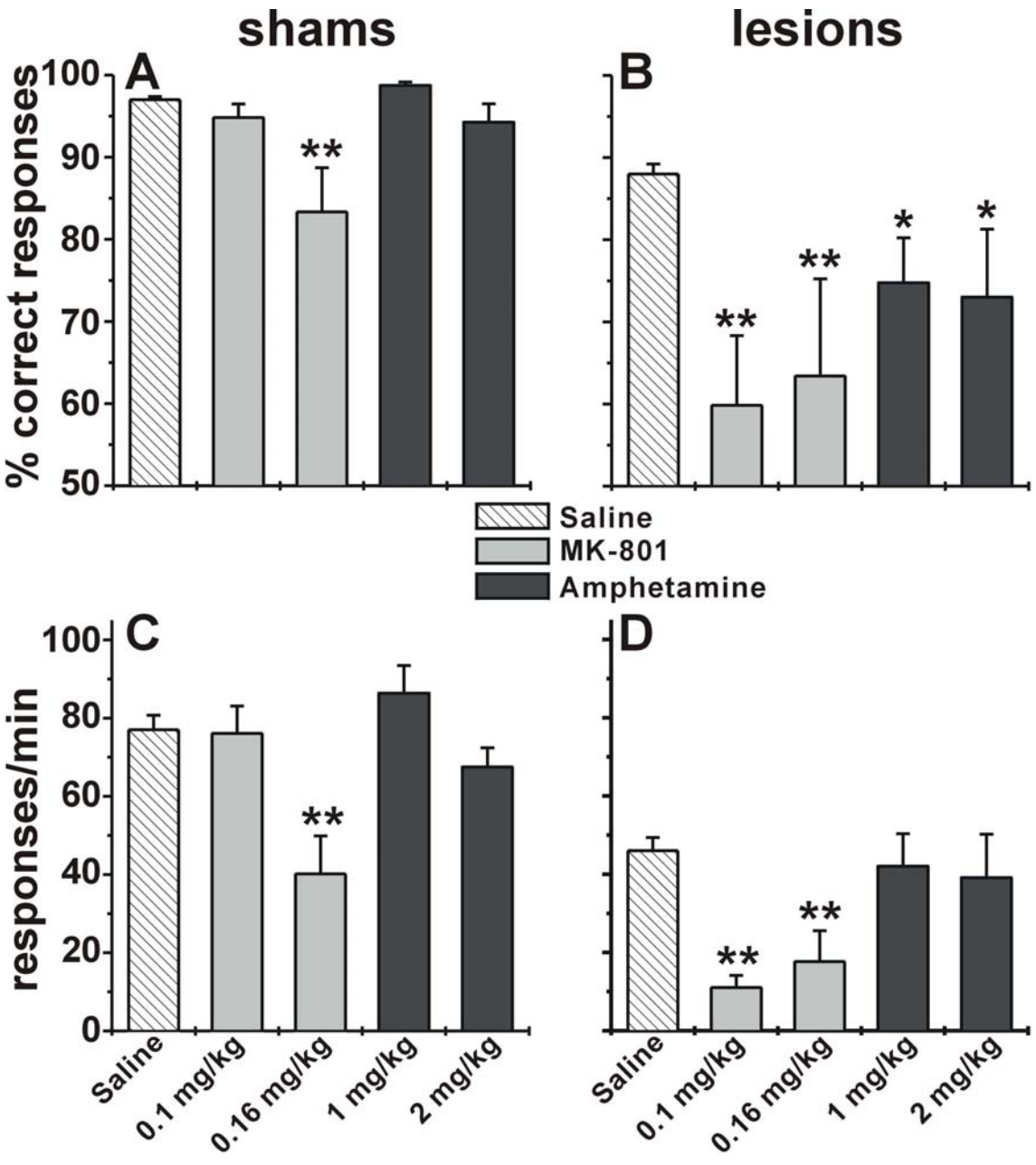

Fig. 11. Performance in the operant discrimination task (ODT) after MK-801 (0.1 and 0.16 mg/kg) or DL-amphetamine (1 and 2 mg/kg) challenge. Bar diagrams present the performance of sham (A and C) and PPTg lesioned (B and D) animals in the ODT. Fascinated bars represent saline treated groups, light gray bars represent MK-801 treated groups and dark gray bars represent DL-amphetamine treated groups. Analyzed parameters were: Accuracy expressed as % correct responses (A and B) and response rate expressed as responses/minute (C and D).*P < 0.05, **P < 0.01 significantly different from saline. Data presented as mean ± S.E.M.

Discussion

In the present study we investigated the effects of DA and GLU in PPTg lesioned rats on performance in an ODT. Lesion itself produced a dramatic decrease in response accuracy and response rate with long-lasting deficits that are even evident after a long period without testing. Moreover, blockade of NMDA receptors by MK-801 treatment showed impairment of accuracy as well as responding in sham-lesioned and more prominent in lesioned animals. Thus resembling the effects induced by the lesion itself. Stimulation of dopamine receptors by amphetamine was, however, without an effect in sham-lesioned animals but impaired accuracy but not responding in lesioned animals.

Some of our findings are in line with earlier studies showing deficits in operant (Alderson, Brown *et al.* 2002; Inglis, Olmstead *et al.* 2000; Inglis, Dunbar *et al.* 1994) and also non-operant discrimination tasks (Inglis, Olmstead *et al.* 2001; Olmstead, Inglis *et al.* 1999) in PPTg lesioned animals. Our data on spontaneous behavior elaborate data from the literature by showing that PPTg lesions produce long lasting deficits in responding to a primary reinforcer. Thus, no recovery was found in the impaired performance of PPTg lesioned animals in an ODT which indicates that no compensation mechanism for PPTg lesion-induced changes exists. Although, sham lesioned rats show a short-term deficit in response rate similar to lesioned rats also it is less pronounced and recovered within one day while that of lesioned rats persists over the whole experiment. The reason behind this is unknown but it can be speculated that it is due to stress of surgery.

The PPTg is anatomically connected to circuitries involved in modulations of motor functions (Semba & Fibiger 1992; Steininger, Rye *et al.* 1992; Hallanger & Wainer 1988; Rye, Lee *et al.* 1988) and also behavioral studies link the PPTg to locomotor processes (Munro-Davies LE, Winter *et al.* 1999; Mogenson, Brudzynski *et al.* 1993; Garcia-Rill E. 1991). Therefore it might be argued that the deficits of PPTg lesioned animals in the ODT are due to motor impairments. However, we did not find any deficits in spontaneous locomotor activity in an open

field in this or in a previous study (Steiniger & Kretschmer 2004). It seems therefore unlikely that the impaired performance in the ODT, seen in the present study, results from motor deficits. Indeed, also other studies do not suggest an effect of the PPTg in motor behavior (Inglis, Allen *et al.* 1994; Olmstead & Franklin 1994). Moreover, coordination of fine motor processes, a parameter that may interfere with responding in the ODT, seems not to be the reason for the deficits of PPTg lesioned rats in the ODT. Alderson et al. (2002) found that PPTg lesions produce impairments in operant responding to a conditioned reinforcer under a progressive ratio schedule only at high, but not at low schedule requirements, suggesting no general deficits in the execution of operant behaviors (Alderson, Brown *et al.* 2002). Also, PPTg lesions produced deficits in a non-operant discrimination task (Inglis, Olmstead *et al.* 2001; Olmstead, Inglis *et al.* 1999), i.e. in a situation in which fine motor processes have no important function.
Another explanation for the deficits seen in the present study might involve deficits in reward perception; (1) because the PPTg receives input from reward related areas, e.g. nucleus accumbens shell and lateral hypothalamus (Steininger, Rye *et al.* 1992; Heimer, Zahm *et al.* 1991), and (2) because a number of studies suggested that reward perception is altered by PPTg manipulations (Olmstead, Munn *et al.* 1998; Lepore & Franklin 1996; Yeomans, Mathur *et al.* 1993). Nevertheless, more recent studies are not in favor of a role of the PPTg in motivation or reward perception (Alderson, Brown *et al.* 2002; Keating & Winn 2002; Olmstead, Inglis *et al.* 1999; Dunbar, Hitchcock *et al.* 1992). Thus, consumption, choice or contrast effects of different sucrose solutions indicated no disruption of primary motivation, reward perception or responding to reward strength after PPTg lesions (Olmstead, Inglis *et al.* 1999; Alderson, Jenkins *et al.* 2001;Keating, Walker *et al.* 2002). Furthermore, PPTg lesion rats perform normally at low schedule requirements in a progressive ratio paradigm and are as fast as controls to collect reward in this task (Alderson, Brown *et al.* 2002) as well as in a radial maze task (Keating & Winn 2002).

It is therefore more likely that the deficits reported in the present study represent an impairment of response selection or attention processes. A concept also discussed by others (Alderson, Brown *et al.* 2002; Keating & Winn 2002; Inglis, Olmstead *et al.* 2001; Inglis, Olmstead *et al.* 2000; Winn 1998). The term 'attention' comprises several sub-processes, which are defined elsewhere (Robbins & Everitt 1995; Sarter & Bruno 2000). One of these sub-processes is 'executive control', which refers to the ability to: 'select and perform the appropriate response to a stimulus' (Robbins & Everitt 1995; Sarter & Bruno 2000; Inglis, Olmstead *et al.* 2001). The comparing of this definition with the term response selection, which refers to the ability to facilitate appropriate and inhibit inappropriate responses, demonstrates the dilemma of an adequate differentiation between attention and response selection processes. The analyzed parameters in the ODT are not sufficient to adequately distinguish these two processes, since a pure deficit in either process would lead to a marked reduction in response accuracy with only little effects on the response rate. Nevertheless, from an anatomical-pharmacological point of view a differentiation seems possible: Response selection processes are linked more to processing of limbic response choice information with basal ganglia sensory-motor information (Horvitz 2002), while attention processes are more linked to thalamocortical projections which regulate the activity state of the cortex (Sarter & Bruno 2000). Thus, the PPTg is on the one hand linked to response selection processes, by affecting DA and GLU systems in BG and limbic systems, and is on the other hand linked to attention processes by affecting thalamocortical projections through its acetylcholinergic innervation of the thalamus (Alderson, Brown *et al.* 2002; Keating & Winn 2002; Inglis, Olmstead *et al.* 2001; Inglis, Olmstead *et al.* 2000; Olmstead, Inglis *et al.* 1999; Winn 1998). Therefore, a response selection deficit caused by PPTg lesions, might be more vulnerable for changes in the DA or GLU system while an attention deficit might be more vulnerable for changes in the ACh system. In this context it is interesting to see that manipulation of the glutamatergic as well as the dopaminergic systems differentially effect the ODT performance as shown in the present study (see below).

2. Drug induced behavior

A. MK-801 treatment

The results of the present study show that blockade of glutamate systems impair response accuracy as well as response rate in the ODT. These findings are in line with other studies: DeVry and Jentzsch (2003) and Cole et al. (1993) found comparable effects after NMDA receptor blockade in a two-lever fixed-ratio operant procedure an operant delayed-matching-to-position task, respectively. It is however new that lesion itself and NMDA receptor blockade resemble in their effects on ODT and that lesioned animals are more sensitive to NMDA receptor blockade. The impairment are more pronounced in the higher dose of MK-801 and are already present at the lower dose. This implies that glutamatergic integrity is necessary in behavioral tasks depending on PPTg functioning. In fact, glutamatergic neurons are described within the PPTg (Clements & Grant 1990) and loss of these neurons might be fundamentally involved in the here described effects. However, possible circuits through which the PPTg exerts its glutamate driven, behavioral modulatory functions are the interconnections with the STR/NAC systems: (1) The PPTg might act as an corticostriatal output station; i.e. directing information (Semba & Fibiger 1992; Steininger, Rye *et al.* 1992) to brainstem motor nuclei and spinal cord (Rye, Lee *et al.* 1988). (2) The PPTg might form a long loop; i.e. directing information back to STR/NAC and related systems (Hallanger & Wainer 1988). In the present study we cannot exclude either possibility, but the finding that the effects of MK 801 - which most probably acts through blocking NMDA receptors in the NAC - is enhanced by PPTg lesions favor the long loop for mediating the here reported effects. Nevertheless, it is obvious that STR/NAC systems are a possible location of glutamatergic PPTg action. In fact, NMDA receptors are described on STR/NAC output neurons which are a possible target of MK-801 actions (Tallaksen-Greene, Wiley *et al.* 1992) and which might be altered due to PPTg lesions. Furthermore, NMDA receptor blockade in the NAC lead to a reduced accuracy and response rate in operant

behavior (Kelley, Smith-Roe et al. 1997). Deficits which are similar to the here reported MK-801 effects, and which are enhanced by PPTg lesions. The exact nature of these deficits is not clear, but it is known that MK-801 acts via the NAC on motor behavior. Therefore, the same mechanism that induces locomotion after MK-801 treatment might also affect ODT performance. The concept that PPTg and GLU systems synergistically modulate behavior through the STR/NAC systems is further underlined by a previous finding, where we showed a synergistic effect of PPTg lesions and MK-801 on exploratory motor behavior in the open field (Steiniger & Kretschmer 2004). Since the ODT performance necessitates a cessation of locomotion in order to maintain continuous responding for food reward, the deficits in the ODT seen after MK-801 treatment might be due to the conflict of an increase in locomotion and continuous responding. This hypothesis would especially explain the dramatic decrease of the response rate after MK-801. Whether the locomotion inducing mechanism of MK-801 is the only cause for the deficits in the ODT is not clear, because cognitive processes might also be altered by MK-801, as well as PPTg lesions. MK-801 is known to affect cognitive processes like learning (Butelman 1989) and also the PPTg was linked to cognitive processes earlier (Winn 1998; Steckler, Inglis *et al.* 1994). Especially, the attenuated accuracy in responding might be due to cognitive deficits rather than locomotor effects. In this respect Mogenson and colleagues described the NAC, as the place of PPTg actions that is an area of limbic-motor integration (Mogenson, Brudzynski *et al.* 1993). Therefore, the influencing information processing in the NAC seems to be the common target of glutamatergic and PPTg action and could underlie the synergistic effect of modulation to both systems.

B. Amphetamine Treatment

In the present study it is shown that stimulation of the DA system does not affect the ODT performance. Although other studies found amplified responding for conditioned reinforcement (Parkinson, Olmstead *et al.* 1999) and dose dependent increase of low and decrease of high rates of responding under a FI schedule of reinforcement (Dews 1958), we did not find such effects after DL-amphetamine administration. Thus, the DA seems not to be important for responding in the ODT. Although, the response rates in the present study might be in a range not affected by DL-amphetamine.

Nevertheless, PPTg lesions reduced the accuracy of responding, but did not affect the response rate in the ODT after DL-amphetamine administration. This suggests a more cognitive deficit, which is most likely driven by modulations of dopaminergic systems. In fact, dopaminergic input to the PPTg area was reported earlier (Steiniger & Kretschmer 2003), but more over the PPTg is described to be a major excitatory input to dopaminergic midbrain regions, i.e. SN and VTA (Oakman, Faris *et al.* 1995; Hallanger & Wainer 1988; Jackson & Crossman 1983). In line, electrical and pharmacological stimulation of PPTg neurons lead to an activation of both VTA and SN neurons (Lokwan, Overton *et al.* 1999; Scarnati & Florio 1997; Kelland, Freeman *et al.* 1993) and to changes of DA transmission in NAC/STR (Chapman, Yeomans *et al.* 1997; Klitenick & Kalivas 1994; Hernandez-Lopez, Gongora-Alfaro JL *et al.* 1992; Niijima & Yoshida 1988). Thus, dopamine receptors in the NAC/STR might be altered due to PPTg lesions, which might influence the behavioral response to systemic DL-amphetamine seen in the present study. Miller and colleagues reported an increased amphetamine dependent DA efflux in PPTg compared to sham lesioned rats (Miller, Forster *et al.* 2002), supporting our concept. Therefore, dopaminergic midbrain neurons are the most likely location of PPTg action on dopamine driven behaviors. Nevertheless, the exact mechanisms underlying the here reported effects after DL-amphetamine treatment is not clear. However, amphetamine is known to produce stereotyped

behaviors through the NAC/STR systems (Kelly, Seviour *et al.* 1975). Stereotyped behaviors often continue repetitively for long periods and, in this sense, resemble the perseverative responding produced by amphetamine in operant settings (Teitelbaum and Derks 1958). Therefore, a possible explanation for the reduced response accuracy in PPTg lesioned rats treated with amphetamine could be an increase in perseverative responding. The rats continue pressing the same lever and stop switching. Nevertheless, we did not find an increase in the response rate, which might be due to the high baseline response rate produced in our setting. On the other hand d-amphetamine is not only known to produce preservation of responding, which leads to augmented response rates, but can also produce increases in response switching (Evenden & Robbins 1983). This bimodal effect is depending on the dose, the context and the probability of the response under control conditions. Perseveration of responding occurred at higher doses (2.3 to 3.2 mg/kg) of d-amphetamine than those producing increased switching. Thus, an alternative explanation of our results might be that PPTg lesioned animals treated with DL-amphetamine switch more often the lever, but keep on responding. In fact our PPTg lesioned animals show a decrease in accuracy of responding, while the response rate was maintained, a pattern which is in line with the above mentioned concept. Since we did not observe this effect in sham lesioned rats, the PPTg lesions seem to induce or amplify the amphetamine effect on switching behavior (Evenden & Robbins 1983). This hypothesis is further corroborated by the findings that PPTg lesions produce changes in both intensity and sort of amphetamine induced stereotypies (Miller, Forster *et al.* 2002; Inglis, Allen *et al.* 1994). Systemic amphetamine injections in PPTg lesioned rats produced orofacial stereotypies (Inglis, Allen *et al.* 1994), which are normally never seen after systemic administration of amphetamine, but are seen after direct dopaminergic stimulation of the ventro-lateral STR (Delfs & Kelley 1990). When such injections were made in PPTg lesioned animals, the stereotypies were longer lasting and more intense (Allen & Winn 1995). In addition, in vivo chronoamperometry revealed an augmentation of DA efflux after a single amphetamine challenge in PPTg lesioned

as compared to sham lesioned rats (Miller, Forster *et al.* 2002). Still, it cannot be conclude that PPTg lesions in general have a synergistic effect with amphetamine treatment. It was shown that PPTg lesions block the acquisition, but not the retention, of amphetamine conditioned place preferences in formerly drug-naive rats (Olmstead & Franklin 1994; Bechara & van-der-Kooy 1989). Moreover, amphetamine blocks exploratory behavior in an open field after amphetamine treatment (Steiniger & Kretschmer 2003) and reduces the sensitization of locomotor responses to repeated amphetamine administrations (Alderson, Faulconbridge *et al.* 2003). Nevertheless, the here reported deficits of PPTg lesioned rats treated with DL-amphetamine are most likely due to PPTg influences on dopaminergic systems, which alters information processing on NAC/STR level and affects response selection mechanisms.

Conclusion

It was shown that the ODT is sensitive to PPTg manipulation. Thus, excitotoxic lesions of the PPTg produces long lasting attenuations in response rate and accuracy of performance in this task. This attenuation of ODT performance, seems not to be the result of motor deficits or alterations in reward perceptions, but is most likely due to PPTg depending alteration of the response selection which is processed through DA/GLU interactions on STR/NAC level. This concept is underlined by the finding of the present study that the accuracy of ODT performance is sensitive to manipulations of the dopaminergic and glutamatergic system in lesioned rats. Furthermore, NAC lesions induce decreased responding and deficits in accuracy in an operant task (Balleine & Killcross 1994), as do PPTg lesions in the present study. And PPTg lesions induce similar impairments in behavioral tasks, i.e. random foraging task or the delayed spatial win-shift task, which were shown to be sensitive to manipulations of the NAC and its afferent (prefrontal cortex) and efferent (ventral pallidum) systems (Keating & Winn 2002;

Floresco, Braaksma *et al.* 1999; Floresco, Seamans *et al.* 1997). These data imply that the deficits of PPTg lesions are due to changes in DA/GLU interactions on NAC/STR level and that the PPTg is part of a long loop circuitry involved in the processing of response selection.

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IV

IV. Steiniger-Brach & Kretschmer Eur J Neurosci

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Different function of pedunculopontine GABA and glutamate receptors in nucleus accumbens dopamine, pedunculopontine glutamate and operant discriminative behavior

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Abstract

The nucleus accumbens, as the main input structure of the ventral basal ganglia loop, is described as a limbic-motor-interface. Dopamine input to nucleus accumbens modulates processing of concurrent glutamate input from limbic structures carrying motor and motivational information. There is evidence that these dopamine/glutamate interactions are fundamentally involved in response selection processes. However, the pedunculopontine tegmental nucleus (PPTg) in the brainstem is connected with limbic structures as well as dopaminergic midbrain areas, which are also projecting to the NAC. Furthermore, behavioral studies implicate the PPTg in complex and motivated behavior. Thus, the PPTg might be involved in motivated behavior by influencing response selection processes on nucleus accumbens level.

In this study we used *in vivo* microdialysis in freely moving rats in order to inhibit (100, 200, 300 and 400 μM baclofen) or stimulate (5, 12.5, 25 or 50 μM AMPA) the PPTg in animals that are performing an operant discrimination task for food reward. The behavioral consequences were correlated with dopamine and glutamate levels in nucleus accumbens and PPTg, respectively.

PPTg inhibition by local $GABA_B$ receptors impaired the response rate and accuracy of performance in the operant discrimination task. PPTg stimulation by local AMPA receptors impaired exclusively the response rate. Both treatments blocked the performance-driven dopamine signal in nucleus accumbens, whereas glutamate in PPTg was enhanced after AMPA administration only.

The data implicate that the PPTg functionally participates in a network of sub-cortical and cortical structures, which is responsible for the execution of motivated behavior and response selection processes.

Introduction

The basal ganglia (BG) are involved in motor-, reward-, emotional- and cognitive processes (Graybiel, 1997; Schmidt and Kretschmer, 1997; Schultz et al., 1997; Kalivas and Nakamura, 1999). They are organized in parallel, but interconnected, cortico-striato-cortical loops (Alexander et al., 1986; Joel and Weiner, 1994). The nucleus accumbens (NAC), the input structure of the ventral BG loop, is discussed as a limbic-motor interface (Mogenson, 1987; Groenewegen et al., 1996). Meso-accumbal dopamine (DA) modulates processing of concurrent glutamate (GLU) input from limbic structures which are carrying, motor and motivational information (Pennartz et al., 1994; Schmidt, 1998; Horvitz, 2002). As a consequence response selection processes are modulated which are responsible for facilitating appropriate and inhibiting inappropriate motor responses (Salamone et al., 1997; Schmidt, 1998; Koch et al., 2000).

The pedunculopontine tegmental nucleus (PPTg) represents - next to the BG output nuclei - an output station where information from the NAC can be linked to motor relevant areas (Heimer et al., 1991; Semba and Fibiger, 1992; Steininger et al., 1992; Groenewegen et al., 1996). Moreover, the PPTg is substantially connected with structures of NAC DA/GLU interactions. It receives DAergic input from the ventral tegmental area (VTA) and GLUergic input from motor cortical and limbic areas (Semba and Fibiger, 1992; Steininger et al., 1992; Steiniger and Kretschmer, 2003). Furthermore, it sends ascending projections containing GLU and acetylcholine to DAergic midbrain nuclei, to GLUergic limbic areas and to the NAC (Jackson and Crossman, 1983; Hallanger and Wainer, 1988; Oakman et al., 1995). Interestingly, the PPTg represents next to cortical and limbic structures the only glutamatergic input to mesoaccumbal DA cells (Sesack et al., 2003). Thus, the PPTg is anatomically integrated into a circuit that modulates functioning of DA/GLU interactions in the NAC. This connectivity links the PPTg to motor and cognitive behaviors (Mogenson, 1987; Steckler et al., 1994; Winn, 1998), and most

probably to response selection processes mediated by DA/GLU interactions (Steiniger and Kretschmer, 2001; Steiniger and Kretschmer, 2004).

This hypothesis is supported by data showing that electrical and pharmacological stimulation of PPTg neurons activate VTA DA neurons (Kelland et al., 1993; Scarnati and Florio, 1997; Lokwan et al., 1999) and enhance DA transmission in the NAC (Niijima and Yoshida, 1988; Klitenick and Kalivas, 1994). Furthermore, we have previously shown that PPTg DA is modulated by local GABA and GLU receptor stimulations (Steiniger and Kretschmer, 2003); and that PPTg driven behavior is depending on the motivational state of animals (Steiniger and Kretschmer, 2004). Thus, PPTg manipulations affected only behavior of active animals that are in a high motivational state, e.g. animals that are exploring a new environment or performing a lever pressing task.

To elucidate the particular function of DA/GLU interactions in motivated behavior modulated by the PPTg, $GABA_B$ or AMPA receptors stimulation was done using reversed microdialysis (Ungerstedt et al., 1982) in animals that were performing an operant discrimination task (ODT) for food reward. The ODT is a task of high motivational salience that is known to be PPTg dependent (Steiniger and Kretschmer, 2001). The behavioral consequences of the pharmacological PPTg manipulations were correlated with DA and GLU levels in the NAC and PPTg, respectively.

Materials and Methods

Animals

All experiments were carried out in male Sprague Dawley rats (Charles River, Sulzfeld, Germany). Before surgery the rats were housed in groups of six to eight in laboratory cages (Pereg; Techniplast 95 x 44 x 21 cm) under standard conditions

(temperature 22 ± 3 °C; light from 0600 to 1800 hours). After surgery the rats were housed individually in laboratory cages (30 x 45 x 30 cm). Food was restricted to approximately 12 g/day including pellet-intake during behavioral training and testing. If necessary animals were fed 1 to 2 h after behavioral testing. Body weights were monitored regularly. Tap water was available ad libitum.
Experiments were performed in accordance with the ethical guidelines regarding the care and use of animals and were approved by the local council of animal care (ZP1/00).

Drugs

The $GABA_B$-receptors agonist (±) baclofen (Sigma, Deisenhofen, Germany) and the GLU receptor agonist α-amino-3-hydroxy-5-methyl-isoxazole-4-prpionic acid (AMPA) (Biotrend RBI, Köln, Germany) were used in doses of 100, 200, 300 and 400 µM and 5, 12.5, 25 and 50 µM, respectively. Drugs were diluted in artificial cerebrospinal fluid (aCSF) containing 147 mM NaCl, 2.5 mM KCl, 1.3 mM $CaCl_2$ and 0.9 mM $MgCl_2$. AMPA was first dissolved in a minimum amount of NaOH and diluted with aCSF to the final concentration. Each day aCSF, as well as AMPA and baclofen solutions were controlled to have a pH of 7.2 ± 0.1.

Operant chamber apparatus

Four operant chambers (24 x 21 x 30 cm) (Med Associates, St. Albans, UK) were used. Each chamber was supplied with a food dispenser, an pellet cup, two retractable levers and two stimulus lights above each retractable lever. The experiments were controlled online by a computer system (SmartControl-Interfaces, MedPC-Software, Med Associates).

Operant procedures

Behavioral protocols were used according to an earlier study (Steiniger and Kretschmer, 2001), with slight modifications in order to adapt the protocol to the microdialysis set up.

Briefly, animals were trained once a day during day-light to perform an ODT with increasing levels of complexity until the final test protocol was reached (Fig. 1) (Details see below). Meeting this criterion, surgery followed. After a recovery-period of minimal 7 days, the training perpetuated (post surgery training-days) until a criterion of 95 % correct responses (accuracy) was achieved for three consecutive days. Subsequently, the microdialysis- and a post-microdialysis-day followed:

An ODT day under the final test protocol was divided into three parts. (1) equilibrium-phase: on training- and post-microdialysis-days, animals remained in the operant chamber for random times between 10 – 240 min; on a microdialysis-day all animals remained 240 min in the operant chamber. Stimulus lights were turned off and no lever was presented. (2) Performing-phase: a fixed duration of 60 min on all days and was subdivided into several ON-periods with random durations between 1 - 2 min, which were separated by OFF-periods with a fixed duration of 10 sec. During ON-periods two levers and one stimulus light above the rewarded lever were presented, while during the OFF-periods levers were rejected and the light was turned off. The rewarded side was randomly chosen for each ON-period and was rewarded under a fixed ratio 10 schedule of reinforcement. Thus, 10 lever presses resulted in the delivery of one 45 mg sucrose pellet (Formula P, P.J. Noyes Company, Inc., Lancaster, USA). Lever presses on the unrewarded lever was counted as false responses, but had no further consequences. (3) Post-performance-phase: the animals remained in the operant chambers - with lights turned off and rejected levers - for random times between 10 - 100 min on training and post-microdialysis-days and for 100 min on a microdialysis-day.

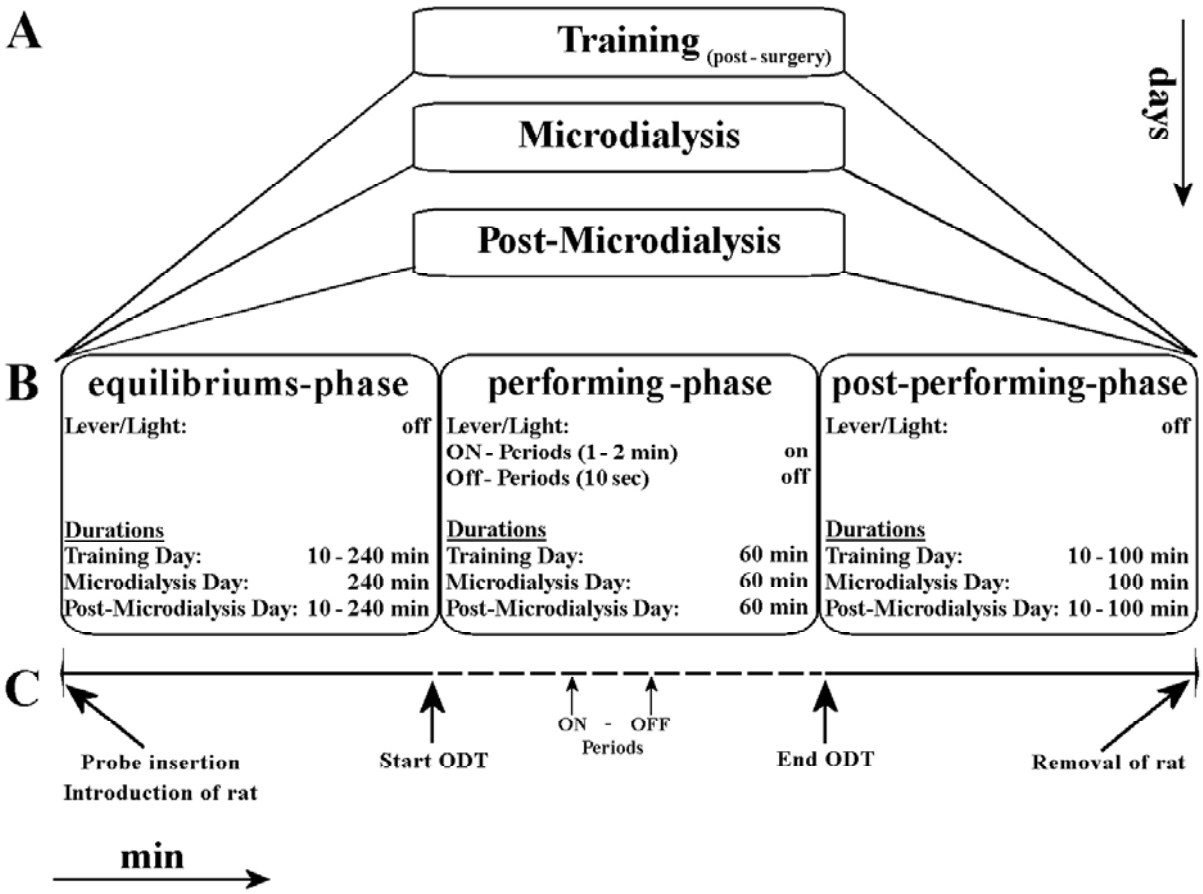

Fig.1 Operant discrimination task (ODT) – Final test protocol used on post surgery training-, microdialysis- and post microdialysis-days. (A) Sequence of ODT days. (B) Details of the different phases: equilibrium-, performing- and post-performing- phase. (C) Timeline representing an ODT day. Note that probe insertion was done only on the microdialysis-day. For details see text.

Surgery

Guide cannulae (CMA microdialysis; Semrau AG, Sprockhövel, Germany) were unilaterally implanted under deep anaesthesia (chloral hydrate, 350 mg/kg i.p.) into the PPTg (posterior -7.8 mm, lateral -1.6 mm, ventral -6.2 mm to bregma according to (Paxinos and Watson, 1998) and NAC (anterior +3.4 mm, lateral –1.5, ventral –6.0 according to (Pellegrino et al., 1986), using standard stereotaxic procedures (described earlier Kretschmer, 1999).

Microdialysis and behavioral analysis

On the microdialysis-day, probes (CMA/12, CMA microdialysis) with an active membrane length of 2 mm were slowly inserted into the PPTg and the NAC and were perfused with aCSF at a flow rate of 1.8 µl/min. Animals were then attached to a tether and placed into operant chambers.

Collection of 20 min fractions started 60 min before the end of the equilibrium-phase. These, three baseline samples (aCSF) were followed by three test samples during the performing-phase, in which animals were locally perfused with baclofen (100, 200, 300 or 400 µM), AMPA (5, 12.5, 25 or 50 µM) or aCSF (control group), while responding in the ODT. Subsequently, during the post-performing-phase five more samples were collected under baseline conditions (Fig. 1)

After the post-microdialysis-day, the brain of each rat was removed and transferred to 4% para-formaldehyde. The localization of the probes was examined in cresyl violet stained frontal brain sections (30 µM).

Biochemical analysis

Monoamines: DA and its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid, as well as 5-hydroxyindole acetic acid were immediately analyzed using reverse-phase high-performance liquid chromatography (Bischoff, Leonberg, Germany) with electrochemical detection (ESA, Bischoff) (described earlier Kretschmer, 1999). Briefly, a HPLC pump (Bischoff) connected to a Prontosil 53 x 3 column (Bischoff), containing a mobile phase of 60 mM NaH_2PO_4, 0.1 mM EDTA, 0.2 mM octanesulfonic acid and 10% methanol (pH 3.73), was used. A detection limit of 0.1 nM DA was routinely achieved.

Excitatory Amino Acids: Glutamate and aspartate were analyzed on a reverse-phase HPLC system with pre-column derivatization using *o*-phtaldialdehyde/mercaptoethanol reagent and flourimetric detection. Briefly, 11µl *o*-phtaldialdehyde/mercaptoethanol were added to 11µl dialysate and injected after a reaction-period of 60 s (at + 4°C) into a HPLC system. An HPLC pump (Dionex pump P580; Dionex, Idstein, Germany) kept a constant flow of the mobile phase

through a Nucleosil 100 C-18 5µm column (Bischoff). Separation was achieved with a mobile phase containing 0.1 M sodium acetate, 6 % methanol and 1.5 % tetrahydrofuran (pH 6.95) as used in earlier studies (Kretschmer et al., 2000). For a rapid cleaning, after each elution of GLU and aspartate, the mobile phase was shifted to 100 % methanol for 1 min, and then back to the mobile phase for the next sample. The flourimetric detection was achieved by a Dionex fluorescence detector RF 2000 with a band-excitation wavelength at ~370 nm and ~450 nm. A detection limit of 10 nM GLU and aspartate was routinely achieved.

Statistics

Comparisons of ODT performance on training-, microdialysis- and post-microdialysis-days were done using a repeated measures one-way analysis of variance (ANOVA) for each group.

Behavioral and neurochemical effects of AMPA, baclofen and aCSF treatment on the ODT performance at the microdialysis-day were assessed by two way ANOVA with dose as a between subject factor and time as a within subject factor for each drug and parameter (accuracy, response rate, GLU and DA).

ANOVAs were followed by the Fischer's LSD-(protected-t)-test if appropriate. The level of significance was set at $p < 0.05$. Data are presented as means ± S.E.M..

Results

Behavior

aCSF

Training- – Microdialysis- – Post-Microdialysis-Days

No difference in the ODT performance of control animals was found, when comparing 5 training-days preceding the microdialysis-day, the microdialysis-day and the post microdialysis-day (Fig. 2) (accuracy: $F_{(6, 4)} = 1.11$ and response rate: $F_{(6, 4)} = 2.39$) (Fig 2 A and B, respectively).

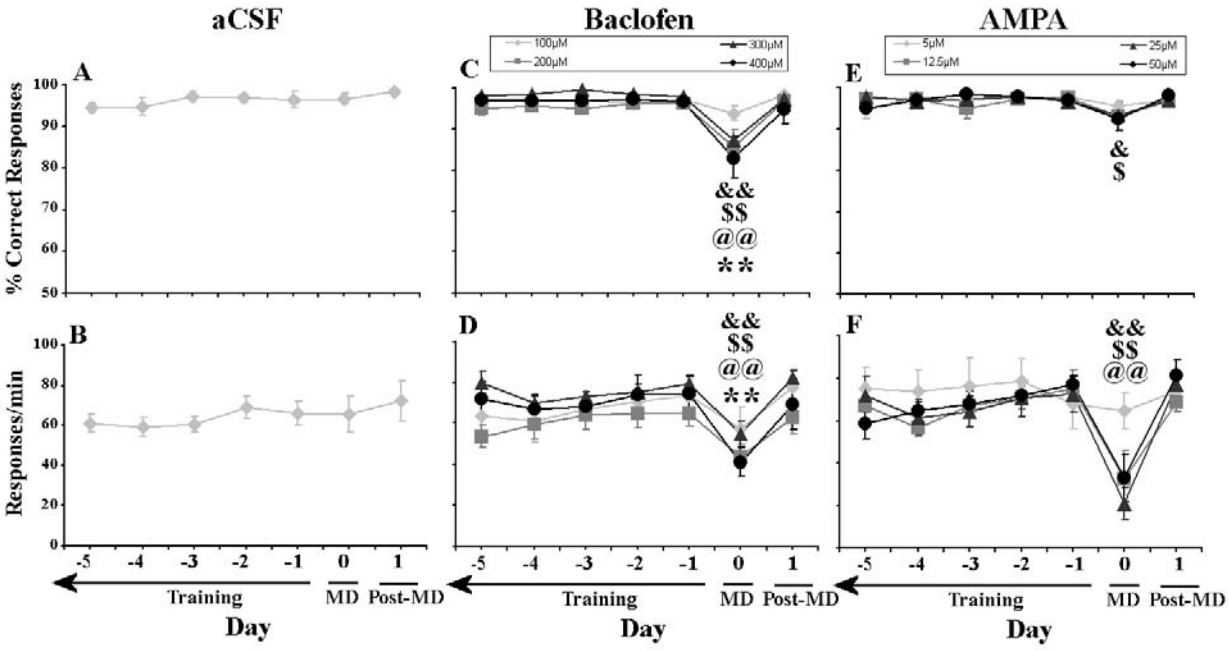

Fig. 2. Performance in the operant discrimination task during training-, microdialysis- (MD) and post-MD-days: Shown are the task accuracy expressed as % correct responses per day (A, C, E) and the response rate expressed as responses/minute (B, D, F), on 5 training-days (day: -5 – -1), the MD- (day: 0) and the post-MD-day (day: 1). Only on the MD-day were animals attached to the MD set up. ACSF (A, B), baclofen (100, 200, 300 and 400 µM) (C, D) and AMPA (5, 12.5, 25 and 50 µM) (E, F) were administered into the PPTg via reversed MD. **p < 0.01 difference

in performance on training-day -1 vs. MD-day of 100 μM baclofen or 5 μM AMPA treated animals. $^{@@}p < 0.01$ difference in performance on training-day -1 vs. MD-day of 200 μM baclofen or 12.5 μM AMPA treated animals. $^{\$}p < 0.05$ and $^{\$\$}p < 0.01$ difference in performance on training-day -1 vs. MD-day of 300 μM baclofen or 25 μM AMPA treated animals. $^{\&}p < 0.05$ and $^{\&\&}p < 0.01$ difference in performance on training-day -1 vs. MD-day of 400 μM baclofen or 50 μM AMPA treated animals. Data presented as mean ± S.E.M.

Baclofen

Training- – Microdialysis- – Post-Microdialysis-Days

PPTg perfusion with 100, 200, 300 and 400 μM baclofen on the microdialysis-day, reduced accuracy (100 μM: $F_{(5, 6)} = 2.8$, $p = 0.03$; 200 μM: $F_{(8, 6)} = 5.21$, $p = 0.0003$; 300 μM: $F_{(4, 6)} = 26.9$, $p < 0.0001$ and 400 μM: $F_{(5, 6)} = 5.57$, $p = 0.0006$) and response rate (100 μM: $F_{(5, 6)} = 4.69$, $p = 0.0018$; 200 μM: $F_{(8, 6)} = 8.98$, $p < 0.0001$; 300 μM: $F_{(4, 6)} = 5.09$, $p = 0.0017$and 400 μM: $F_{(5, 6)} = 4.28$, $p = 0.0032$) as compared to the 5 training-days and the post-microdialysis-day (Fig 2 C and D, respectively).

Microdialysis-Day

A more detailed analysis of the microdialysis-day, i.e. separating performance during the 60 min ODT into 20 min bins is shown in Fig. 3.

Local PPTg baclofen administration dose-dependently attenuated accuracy and number of responses of ODT performance as compared to control animals. Comparing 100, 200, 300 and 400 μM baclofen and aCSF treated animals, ANOVA revealed dose effects ($F_{(4, 96)} = 17.2$, $p < 0.0001$) in accuracy as well as dose ($F_{(4, 96)} = 14$, $p < 0.0001$) and time ($F_{(2, 23)} = 16.8$, $p < 0.0001$) effects in number of responses in ODT performance (Fig. 3).

Post hoc analysis of the accuracy data revealed no difference between control and 100 μM baclofen treatment in all three bins. However, 200 and 300 μM baclofen

reduced the accuracy as compared to aCSF treatment, in the second and the third bin, and 400 μM baclofen in all three bins of the ODT. In addition, in the last bin of the ODT 300 and 400 μM baclofen significantly decreased the accuracy of ODT performance as compared to 100 μM baclofen treated animals (Fig. 3A).

Post hoc analysis of the number of responses revealed attenuation for all treatments in responding in the last bin as compared to the first bin of the ODT. No dose effect was found between aCSF and 100 μM baclofen treatment. However, 200 μM baclofen reduced the responses as compared to aCSF treatment in the second, 300 μM in the second and third and 400 μM in all three time-bins of the ODT. In addition, 200, 300 and 400 μM baclofen treated animals were significantly more effective in reducing the number of responses as compared to 100 μM baclofen treated animals in the second bin (Fig. 3B).

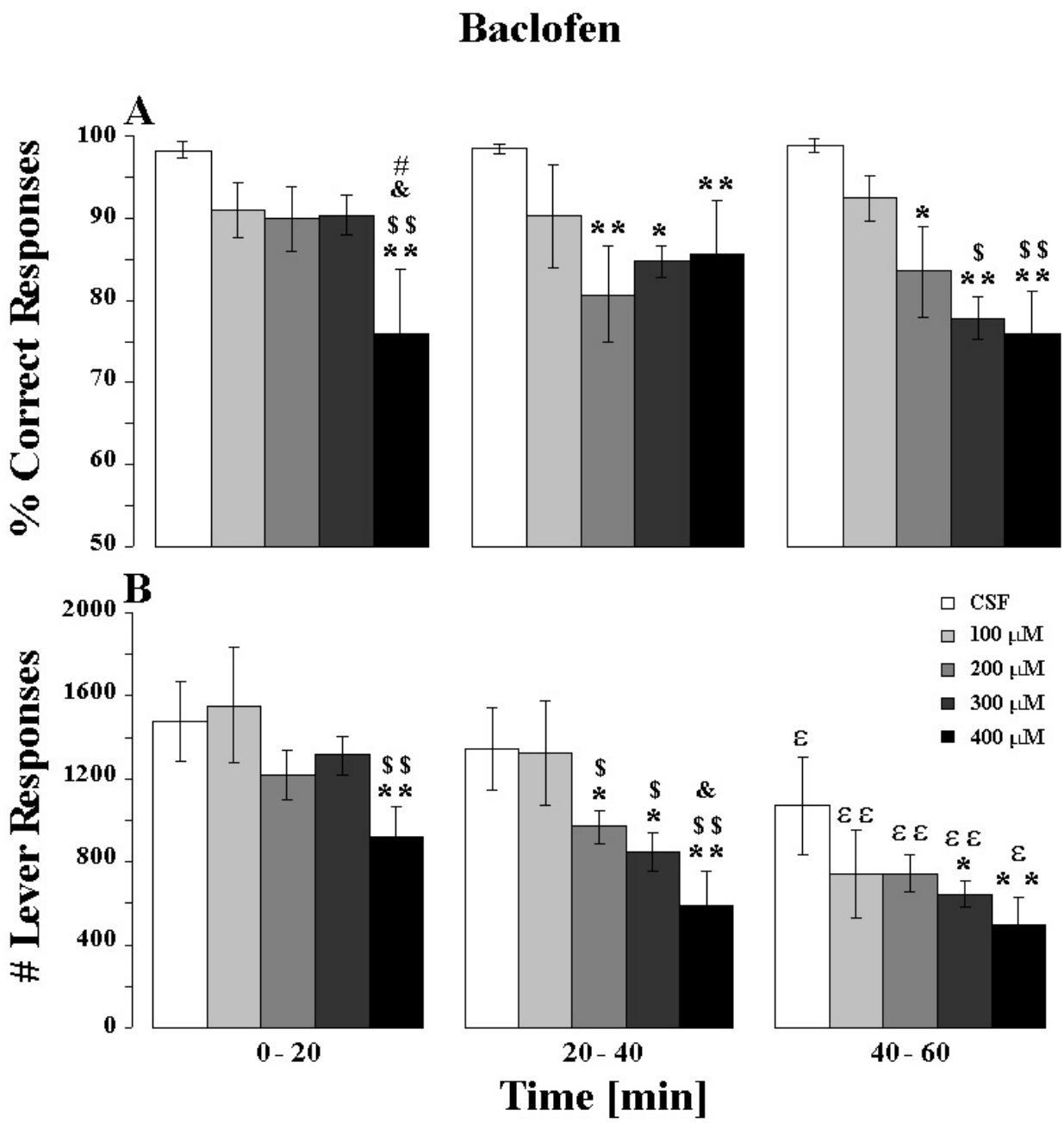

Fig. 3 Baclofen effects on the performance in the operant discrimination task during the microdialysis-day. Data were analyzed in 20 min bins that correspond to the microdialysis sampling (Fig. 5). (B) Number of response rate. (A) Task accuracy expressed as % correct responses. *$p < 0.05$, **$p < 0.01$; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\&}p < 0.05$ and $^{\#}p < 0.05$ dose effects: difference to aCSF, 100, 200 and 300 µM baclofen, respectively. $^{\varepsilon}p < 0.05$, $^{\varepsilon\varepsilon}p < 0.01$ time effects: difference to the same treatment in the first time-bin, respectively. Data presented as mean ± S.E.M.

AMPA

Training- – Microdialysis- – Post-Microdialysis-Days

PPTg perfusion with 12.5, 25 and 50 µM, but not with 5 µM AMPA on the microdialysis-day reduced the response rate (5 µM: $F_{(3, 6)} = 0.83$; 12.5 µM: $F_{(4, 6)} = 6.81$, $p = 0.0003$; 25 µM: $F_{(4, 6)} = 24.3$, $p < 0.0001$ and 50 µM: $F_{(5, 6)} = 10.5$, $p < 0.0001$) of ODT performance (Fig. 2 F). However, only the high doses (25 and 50 µM AMPA) slightly affected the accuracy of ODT performance ($F_{(4, 6)} = 2.85$, $p = 0.031$ and $F_{(5, 6)} = 2.76$, $p = 0.029$, respectively) (but this has to be seen in respect to the more detailed analysis (20 min bins) below), while the low doses (5 and 12.5 µM) had no effect on this parameter ($F_{(3, 6)} = 1.08$ and $F_{(4, 6)} = 0.98$, respectively) (Fig. 2 E).

Microdialysis-Day

Detailed analysis of accuracy and number of responses in 20 min bins indicated an effect of AMPA administration most exclusively in number of responses. ANOVA of the ODT performance of 5, 12.5, 25, and 50 µM AMPA and aCSF treated animals revealed dose ($F_{(4, 72)} = 26.1$, $p < 0.0001$, respectively) and time effects ($F_{(2, 17)} = 16.3$, $p = 0.0002$, respectively) (Fig. 4).

Post hoc analysis revealed an effect on accuracy for 25 and 50 µM treatment in the last bin as compared to aCSF (Fig. 4A). Nevertheless, it is important to note that the number of responses of animals in both dose groups at the last bin was close to 0 (Fig. 4 B), making a current conclusion for a deficit in accuracy unlikely.

Post hoc analysis of the number of responses revealed a dose dependency for AMPA treatment. 12.5, 25 and 50 µM decreased responding in the ODT as compared to aCSF treatment in all bins while 5 and 12.5 µM were effective in the second and third bin, only. 25 and 50 µM AMPA treatment was also significantly more effective as compared to 5µM AMPA treatment in the second and third bin (Fig. 4 B).

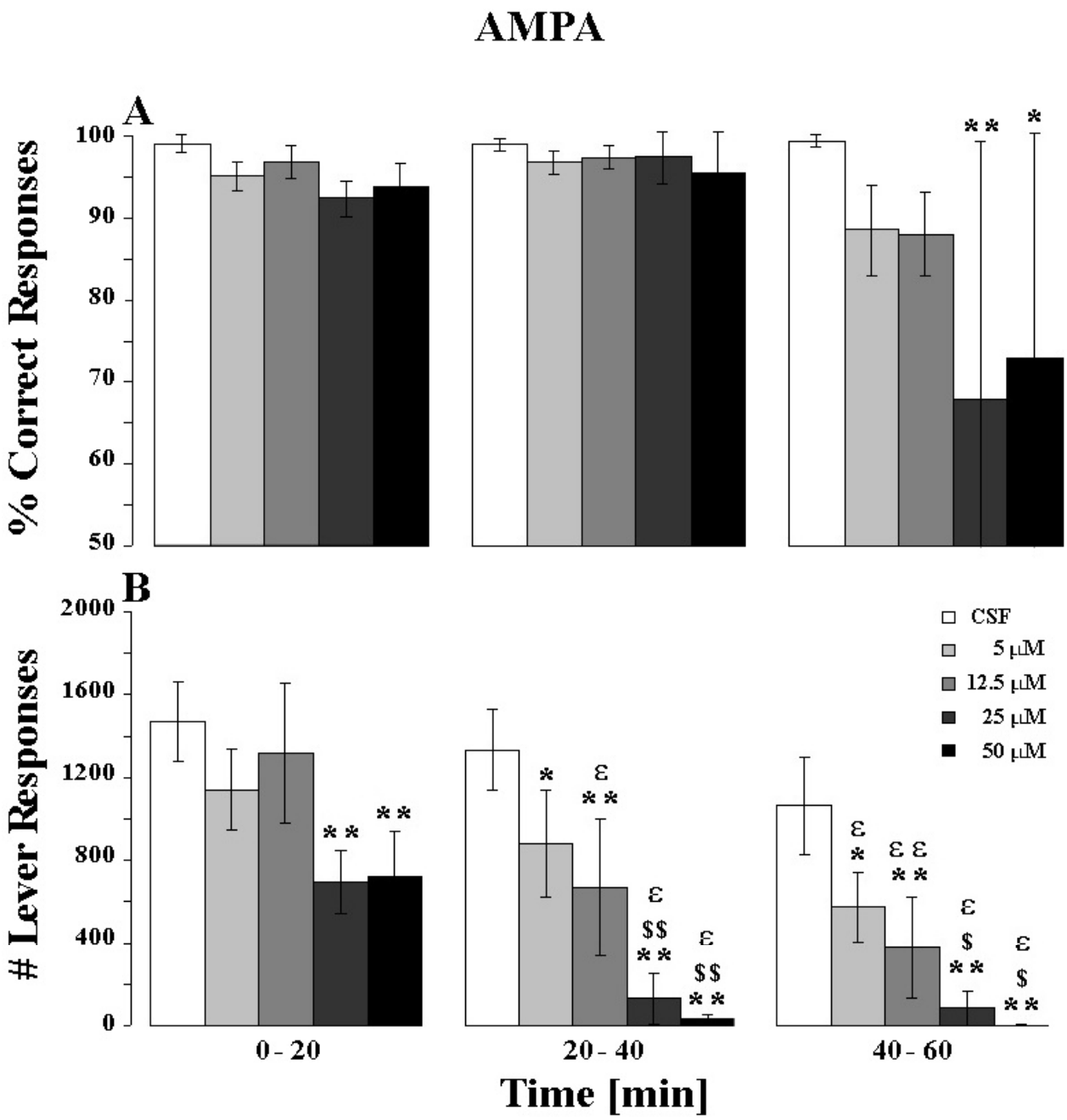

Fig. 4 AMPA effects on the performance in the operant discrimination task during the microdialysis-day. Data were analyzed in 20 min bins that correspond to the microdialysis sampling (Fig. 5). (B) Number of responses. (A) Task accuracy expressed as % correct responses. *$p < 0.05$, **$p < 0.01$; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ and, 5 µM and, respectively. $^{\varepsilon}p < 0.05$, $^{\varepsilon\varepsilon}p < 0.01$ time effects: difference to the same treatment in the first time-bin, respectively. Data presented as mean ± S.E.M.

Neurochemistry

aCSF

In control animals no effect was found on GLU levels in the PPTg, but DA levels ($F_{(10, 5)} = 6.69$, $p < 0.0001$) in the NAC were enhanced during the ODT (Fig. 5 A and B, respectively).

Baclofen

Baclofen in the concentrations of 100, 200, 300 and 400 μM was perfused in the PPTg of animals simultaneously performing in the ODT. No differences over time in PPTg GLU levels were found in these animals, as assessed by two-way ANOVA. Nevertheless, a dose effect was found ($F_{(3, 207)} = 14.9$, $p < 0.0001$) (Fig. 5C).

In the same animals, differences in time ($F_{(10, 63)} = 42.8$, $p < 0.0001$) and dose ($F_{(3, 192)} = 16.6$, $p < 0.0001$) were found in DA levels in the NAC. In post hoc analysis a negative correlation between the DA increase during the ODT and the baclofen concentration was obtained. Later on, a positive correlation between the DA decrease in the last three samples and the baclofen concentration was found (Fig. 5D).

AMPA

AMPA in the concentrations of 5, 12.5, 25 and 50 μM was perfused in the PPTg of animals simultaneously performing in the ODT. Two way ANOVA revealed time ($F_{(10, 52)} = 8.03$, $p < 0.001$) and dose ($F_{(3, 159)} = 11$, $p < 0.0001$) differences in GLU levels in the PPTg. In post hoc analysis a strong increase in GLU levels correlating with the treatment was found for 25 and 50 μM AMPA, whereas 5 and 12.5 μM AMPA produced no significant difference (Fig. 5E).

In the same animals, time ($F_{(10, 54)} = 4.26$, $p = 0.0004$) and dose ($F_{(3, 165)} = 13.2$, $p < 0.0001$) differences were found in DA levels in the NAC. In post hoc analysis a negative correlation between the DA increase during the ODT and the baclofen concentration was found (Fig. 5F).

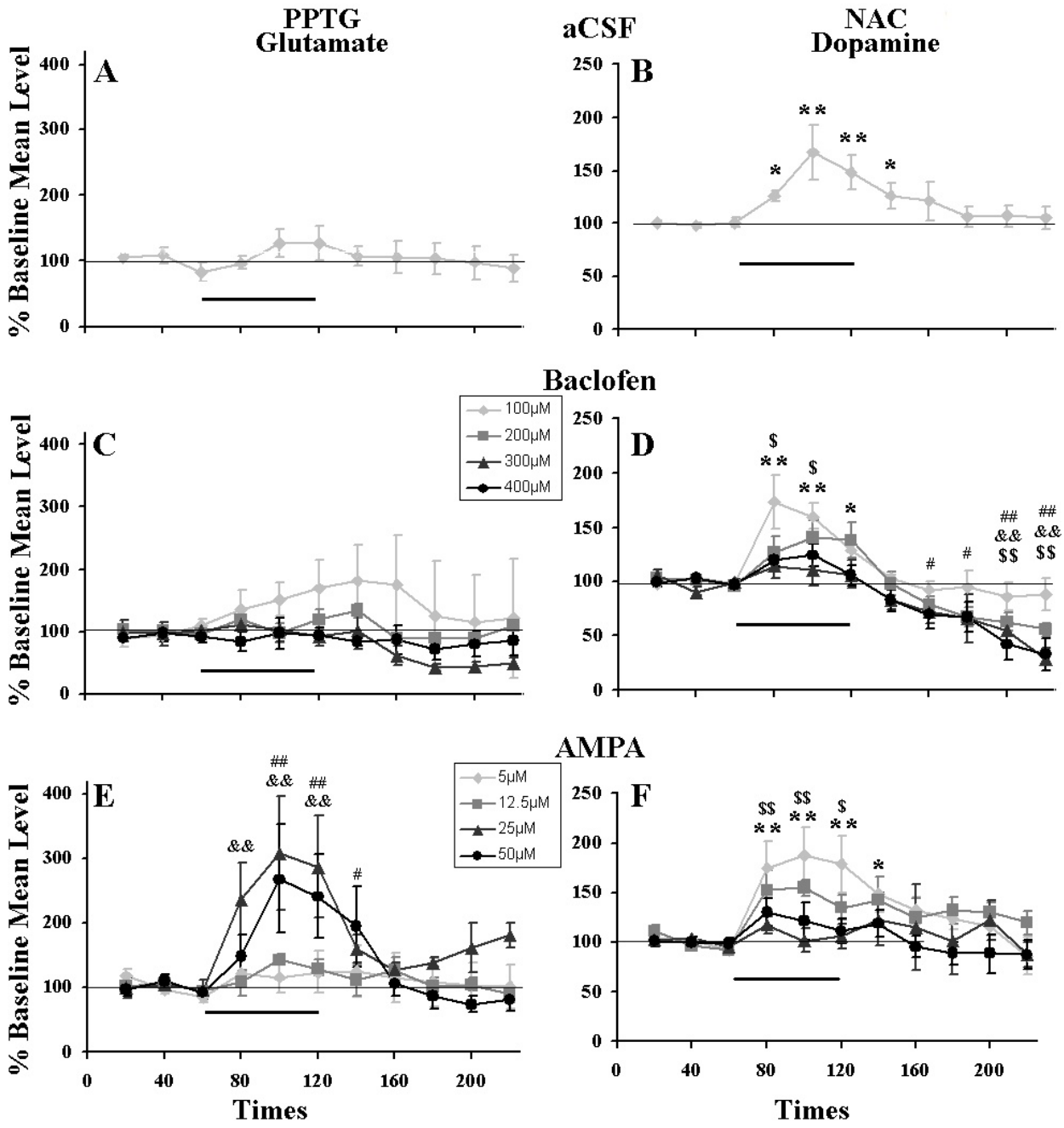

Fig. 5. Nucleus accumbens (NAC) dopamine (DA) (B, D, F) and pedunculopontine tegmental nucleus (PPTg) glutamate (GLU) levels (A, C, E) on the microdialysis-day (see Fig 2.). (A, B) Effects of the operant discrimination task (ODT) on GLU in PPTg and DA in NAC under aCSF conditions. (C, D) Effects of the ODT and baclofen infusions (100, 200, 300 and 400 µM) into the PPTg. (E, F) Effects of the ODT and AMPA infusions (5, 12.5, 25. 50 µM) into the PPTg. Black bars indicate time of behavioral and pharmacological manipulations. Data are presented as percentage of the

basal mean level ± SEM. *p<0.05, **p < 0.01 significant differences for aCSF, 5 µM AMPA and 100 µM baclofen data versus basal mean levels, respectively. $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ significant differences for 12.5 µM AMPA and 200 µM baclofen data versus basal mean levels, respectively. $^{\&\&}p < 0.01$ significant differences for 25 µM AMPA and 300 µM baclofen data versus basal mean levels, respectively. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ significant differences for 50 µM AMPA and 400 µM baclofen data versus basal mean levels, respectively. Data presented as mean ± S.E.M.

Discussion

In the present study we used *in vivo* microdialysis to investigate the effects of local PPTg inhibition or stimulation on performance in the ODT and on DA and GLU levels in the NAC and PPTg, respectively.

In control animals, it was shown, that tethering the animals for microdialysis purposes had no consequences on the performance in the ODT. Nevertheless, the ODT performance itself elevated DA in NAC, but not GLU in PPTg. Moreover, PPTg inhibition by $GABA_B$ receptor stimulation using baclofen infusion led to dose dependent impairments in response rate and accuracy. These effects were accompanied by a dose-dependent blockade of performance-driven DA in NAC (which was found in control animals), but did not affect GLU in PPTg. PPTg stimulation by local AMPA receptor stimulation using AMPA led to a dose-dependent blockade of the response rate. This blockade correlated with a dose dependent blockade of the performance-driven DA increase in NAC (which was found in control animals) and with an increase of GLU on PPTg level.

The DA increase found in control animals - i.e. during ODT performance without pharmacological manipulations - are consistent with previous data demonstrating that lever pressing for food reinforcement on continuous or variable interval reinforcement schedules, increase DA release or metabolism in the NAC (Hernandez and Hoebel, 1988; McCullough et al., 1993; Salamone et al., 1994; Kiyatkin and Gratton, 1994; Sokolowski et al., 1998). Moreover, Sokolowski and colleagues found a positive correlation between response rate and DA

enhancement, but no correlation with the quantity of consumed pellets. Also, electrophysiological and voltammetric studies examining operant performance reported that the period of time in which a lever pressing occurred was generally accompanied by increases in VTA- or DA-related voltammetric activity (Nishino et al., 1987; Kiyatkin and Gratton, 1994). Although the electrophysiological data demonstrate that the activity of the DA neurons do not correlate with the motor act of lever pressing, it is obvious that DA in the NAC is generally related to the instrumental-phase of motivated behavior - a phase that is driven by motor activity. Therefore, we expect that DA in NAC correlates rather with the response rate than with the accuracy of ODT performance (see below).

Neurochemistry

GABAergic afferents to the PPTg arise from different nuclei of the BG, e.g. globus pallidus, ventral pallidum, substantia nigra and NAC (Oertel and Mugnaini, 1984; Heimer et al., 1991; Semba and Fibiger, 1992; Steininger et al., 1992) and act via $GABA_B$ receptors in the PPTg (Chu et al., 1990). Thus, the decrease of DA after infusion of baclofen into the PPTg is most likely the result of local $GABA_B$ receptors which are normally targeted from BG afferents. This is in line with an earlier study in which we showed that $GABA_B$ receptor activation in the PPTg decreased intra-PPTg DA levels (Steiniger and Kretschmer, 2003). The present results elaborate these findings by showing that also mesoaccumbal DA is inhibited by GABAergic inhibition of the PPTg. Interestingly, this inhibition did not only counteract the performance-driven DA increase, but lead to a sustained decrease of DA below baseline value in the late phase of the experiment (Fig. 5D). This decrease is most likely mediated by a direct PPTg – VTA – NAC pathway. In line, direct excitatory PPTg - VTA projections have been described (Kalivas, 1993; Lavoie and Parent, 1994; Oakman et al., 1995; Parent et al., 1999). Furthermore, electrical and pharmacological manipulations of PPTg neurons alter activity of VTA neurons (Kelland et al., 1993; Scarnati and Florio, 1997; Lokwan et al., 1999) and DA transmission in the NAC (Niijima and Yoshida, 1988; Klitenick and

Kalivas, 1994). While this is a straightforward explanation for the baclofen effects on DA levels in the NAC, the decrease of DA in NAC and increase of GLU in PPTg after PPTg AMPA infusion are more surprising:

Known GLUergic afferents of the PPTg come mainly from the subthalamic nucleus (STN), and the prefrontal cortex (PFC) (Semba and Fibiger, 1992; Steininger et al., 1992). Furthermore, AMPA receptor subunits are expressed in PPTg neurons (Inglis and Semba, 1996). Thus, the decrease of DA in NAC and the increase of GLU in PPTg are most likely due to the activation of these AMPA receptors, which are normally targeted from STN and PFC afferents. Contrary to the results of the present study, local PPTg AMPA infusion previously acted as a potent enhancer of PPTg activity and increased DA in PPTg (Steiniger and Kretschmer, 2003). In addition, electrical as well as pharmacological stimulations of these neurons by a GABA antagonist, a μ receptor agonist and kainate enhance DA transmission in the NAC via activation of VTA neurons (Niijima and Yoshida, 1988; Klitenick and Kalivas, 1994). Thus, alternative mechanisms must underlie the decrease of DA in NAC after local AMPA receptor stimulation in PPTg: (1) AMPA receptors might mainly be expressed on GABAergic neurons within the PPTg resulting in a reduced mesoaccumbal DA transmission after PPTg AMPA receptor stimulation. No data are available on the exact expression of AMPA receptors in the PPTg, but the existence of GABAergic neurons that is co-expressed with cholinergic and GLUergic neurons have been described (Ford et al., 1995). Furthermore, it was shown that 30 - 40 % of anterogradely labeled terminals from the PPTg are GABA immunoreactive and have synaptic contact with DAergic VTA neurons (Charara et al., 1996). Nevertheless, this concrete expression of AMPA receptors would be contradictory to privious findings, which showed a DA elevation in PPTg after local AMPA administration (Steiniger and Kretschmer, 2003). (2) AMPA receptor expressing PPTg neurons might specifically target GABAergic interneurons in the VTA. Thus, excitation of these neurons would reduce mesoaccumbal DAergic transmission. Non-DA interneurons in the VTA have been described earlier (Beart and McDonald, 1980; Steffensen et al., 1998). And at least cholinergic PPTg

neurons project to non-DA neurons in the VTA (Garzon et al., 1999). Nevertheless, also indirect evidences for a GLUergic projection to VTA non-DA neurons exist: electrophysiological and pharmacological studies demonstrate a GLUergic modulation of these neurons - presumably from PPTg and PFC. Moreover, NMDA, AMPA and kainate receptors are located on VTA non-DA neurons and they are activated by glutamate receptor agonists (Kalivas et al., 1989; Seutin et al., 1990; Wang and French, 1995). And local or systemic applications of NMDA receptor antagonists reduce the firing rate and spiking of VTA non-DAergic neurons (Churchill et al., 1992). (3) The difference in PPTg- and NAC DA transmission might be related to the difference found in the PFC-and NAC-DA systems. In fact, DAergic activities in PFC and NAC are negatively correlated with each other, as are the DA levels in these regions to the behavioral output (for review see Tzschentke and Schmidt, 2000; Sesack et al., 2003). Thus, intra-PFC administration of amphetamine or apomorphine decrease DA turnover in the NAC, whereas DA antagonists have the opposite effect (Louilot et al., 1989; Jaskiw et al., 1991; Thompson and Moss, 1995). In line, PFC DA depletion increases DA in NAC (Louilot et al., 1989). The relationship of PPTg and PFC might, however, be more than just a resemblance in reactivity. Garzon and colleagues reported that PPTg neurons terminate on VTA DA neurons that have similar DA uptake characteristics as DA neurons projecting to the PFC (Garzon et al., 1999). Thus, PPTg stimulation via AMPA receptors could selectively increase DA transmission in the PFC. This effect is known to be negatively correlated with DA levels in the NAC (see above) and fits nicely to the present data.

Furthermore, the involvement of the PFC (see above (3)) could also explain the elevated PPTg GLU levels we found after PPTg AMPA receptor stimulation. GLUergic PFC projections to the PPTg have been described (Semba and Fibiger, 1992; Steininger et al., 1992). In line, the main DA receptors localized in the PFC are D1 and D5 subtypes (Smiley et al., 1994; Bergson et al., 1995). Still, the net effect of DA on GLUergic PFC neurons is not clear. On the one hand electrophysiological and pharmacological studies confirm excitatory D1 receptor

action on GLUergic pyramidal cells in the PFC (Geijo-Barrientos and Pastore, 1995; Yang and Seamans, 1996; Heinze et al., 1998; Goldman-Rakic et al., 2000; Seamans et al., 2001), on the other hand older studies report of DA driven inhibitory effects on GLUergic PFC neurons (Mora et al., 1976; Bunney and Aghajanian, 1976; Mantz et al., 1988). On the basis of the discussed data, the elevated GLU in PPTg found after AMPA infusion might derive from DA action in the PFC. An alternative source for GLU in the PPTg is the STN. Also the STN is linked to the PPTg. Thus STN activity could be modulated directly, through reciprocal connections (Hallanger and Wainer, 1988) or indirectly, via striatal or cortical areas (Parent and Hazrati, 1995).

Behavior

The behavioral effects of PPTg inhibition by baclofen elaborate previous findings, which demonstrated that PPTg lesions impair the performance in operant (Inglis et al., 1994; Inglis et al., 2000;Steiniger and Kretschmer, 2001; Alderson et al., 2002) and also non-operant discrimination tasks (Olmstead et al., 1999; Inglis et al., 2001). Inglis and colleagues showed that PPTg lesioned rats are able to acquire responding to a conditioned reinforcer, but cannot discriminate between rewarded and non-rewarded levers (Inglis et al., 1994; Inglis et al., 2000). In line, we have previously shown that PPTg excitotoxic lesions produces long lasting attenuations in rate and accuracy of ODT responding. The same study revealed that GLUergic as well as DAergic manipulations potentiate the deficit in performance of lesioned animals in this task (Steiniger and Kretschmer, 2001). Therefore, deficits induced by PPTg lesions are most likely due to alterations in GLU/DA interactions, leading to deficits in response selection processes. This hypothesis is underlined by investigations examining consumption-, choice- or contrast-effects of different sucrose solutions, indicating that PPTg lesions neither disrupt primary motivation nor the ability to evaluate or respond to changes in reward strength, but block the appropriate response choice (Olmstead et al., 1999; Alderson et al., 2001). Also, in concert, Inglis, Winn and colleagues discussed that PPTg lesion deficits affect in

particular the processing of limbic information related to response selection with BG sensory-motor information (Inglis et al., 1994; Winn, 1998; Alderson et al., 2001; Keating and Winn, 2002). Thus, PPTg inhibition by $GABA_B$ receptor activation in the present study, seems to result in response selection deficits, as shown by attenuation of response rate and accuracy in the ODT.

Interestingly, AMPA receptor stimulation in PPTg also decreases the response rate as does the PPTg inhibition, but, in contrast, no effect was found on response accuracy. Moreover, both manipulations decreased DA in NAC, but in contrast to $GABA_B$ receptor stimulation, AMPA receptor stimulation did also increase GLU in the PPTg.

Thus, on the one hand there seems to be a correlation between the decrease in response rate and the blockade of DA in NAC. This is in line with data linking DA in NAC especially to the instrumental-phase of motivated behavior as outlined above (Hernandez and Hoebel, 1988; McCullough et al., 1993; Salamone et al., 1994; Kiyatkin and Gratton, 1994; Sokolowski et al., 1998). On the other hand, the lack of an accuracy alteration after PPTg AMPA receptor stimulation seems to correlate with the increase in GLU levels in the PPTg. Therefore, the GLU increase might be a compensation signal that could derive from the PFC or STN as discussed in the neurochemistry section. A large body of evidence exists that implicate the PFC in higher cognitive and executive functions (as review see Wood and Grafman, 2003). Most interestingly in this regard are deficits in discriminative accuracy (which might be close to the accuracy parameter in the present study) of bilateral PFC- (Muir et al., 1996), as well as STN- (Baunez and Robbins, 1997) and PPTg-lesioned rats (Inglis et al., 2001) performing the five choice serial reaction time task. Furthermore, PFC lesions impair performing in a delayed spatial win-shift task (Floresco et al., 1997), as did PPTg lesions (Keating and Winn, 2002); and recently it was shown that the cortico-subthalamic interaction is crucial for the execution of discrimination tasks (Chudasama et al., 2003). Thus, PFC and STN seem to share functional properties in the discrimination task, and project via GLUergic efferents to the PPTg. Therefore, elevated GLU levels found in the

present study can derive from PFC as well as STN. From a functional point of view it can be speculated that this GLU signal might compensate a response accuracy deficit seen after PPTg-, but also PFC- and STN-manipulations.
The interaction of the PPTg with the PFC and STN implicates its participation not only in sensorimotor functions but also in higher-order cognitive processing, like response selection processes.

Conclusion

The present data demonstrate that PPTg inhibition via local $GABA_B$ receptors impair the response rate and accuracy of ODT performance, whereas PPTg stimulation via AMPA receptors impair exclusively the response rate. Furthermore, both treatments block the performance-driven DA signal in NAC, however, an increase in PPTg GLU levels was only present after AMPA administration.

Thus, $GABA_B$ and AMPA receptors in the PPTg are linked to different mechanisms modulating DA transmission in NAC. While $GABA_B$ receptors are most likely effective via direct excitatory connections to DAergic mesoaccumbal neurons; AMPA receptors might be linked directly to the VTA by GABAergic PPTg neurons or GABAergic VTA interneurons or indirectly via mechanisms involving the PFC. Furthermore, the DA modulations in NAC which were induced by both treatments, are most likely related to deficits in the instrumental phase of motivated behavior – affecting mainly response rate. The GLU signal after AMPA infusion, on the other hand, might be linked to a compensation mechanism for cognitive aspects of the task – affecting mainly response accuracy. This compensation signal is most likely PFC- or STN-driven.
Thus, the present data implicate the PPTg in a network that involves sub-cortical and cortical structures and is responsible for the execution of motivated behavior.

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Abbreviations

aCSF, cerebrospinal fluid

AMPA, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid

ANOVA, analysis of variance

BG, basal ganglia

DA, dopamine

GABA, gamma-amino-butyric-acid

GLU, glutamate

HPLC, high-performance liquid chromatography

NAC, nucleus accumbens

NMDA, N-methyl-D-aspartate

ODT, operant discrimination task

PFC, prefrontal cortex

PPTg, pedunculopontine tegmental nucleus

S.E.M., standard error of the means

STN, subthalamic nucleus

VTA, ventral tegmental nucleus

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4. Ergebnisse: Tabellarische Zusammenfassung

Tabelle 1: Zusammenfassung der verhaltensrelevanten Ergebnisse

Behandlung	ODT		*open field*		Mikrodialyse		
	RR	RG	Exp	Lok	NAC/DA	PPTg/DA	PPTg/GLU
[1] Saline (i.p.)	↓	↓	∅	∅	/	/	/
[2] *Sham* + MK-801 (i.p.)	↓	↓	∅	↑	/	/	/
[2] Läsion + MK-801 (i.p.)	↓↓	↓↓	∅	↑↑	/	/	/
[2] *Sham* + Amphet. (i.p.)	∅	∅	↑	↑	/	/	/
[2] Läsion + Amphet. (i.p.)	∅	↓	∅	∅	/	/	/
[3] PPTg-Inh. (Baclofen)**	↓	↓	/	∅	↓↓	↓	∅
[3] PPTg-Stim (AMPA)***	↓↓	∅	/	∅	↓↓	↑	↑

[1] PPTg-Läsion gegen *Sham*, [2] Substanz gegen Saline, [3] Substanz gegen CSF

* Kontrollgruppe im ODT, ** Lokale PPTg-Inhibition mit Baclofen, *** Lokale PPTg-Stimulation mit AMPA

ODT: Verhaltensparameter gemessen im operanten Diskriminierungstest: RR: *response*-Rate, RG: *response*-Genauigkeit;

Open field: Verhaltensparameter gemessen im *open field*: Exp: explorative Parameter, Aufrichten und Aktivität in der Mitte im *open field test*, Lok: Lokomotion

Mikrodialyse: Neurochemische Parameter gemessen mit der Mikrodialyse/HPLC, NAC/DA: Dopamin im Nucleus Accumbens, PPTg/DA: Dopamin im Pedunculopontinen Tegmentalen Nucleus, PPTg/GLU: Glutamat im Pedunculopontinen Tegmentalen Nucleus.

↑ Erhöhung im Vergleich zu den Kontrolltieren

↓ Reduzierung im Vergleich zu den Kontrolltieren

∅ Keine Veränderung im Vergleich zu den Kontrolltieren

/ Parameter nicht gemessen oder Experiment nicht durchgeführt

5. Diskussion

In dieser Arbeit wurden die neurochemischen Interaktionen zwischen dem PPTg und der ventralen Basalganglienschleife sowie die Funktion dieser Interaktionen bei der Modulation von Verhalten untersucht.

In den oben einzeln aufgeführten Arbeiten wurde gezeigt, dass: (a) der PPTg mit dem DA-System interagiert (Manuskript I), (b) über den PPTg induzierte Verhaltensmodulationen vom motivationalen Status des Tieres abhängen (Manuskript II), (c) der PPTg insbesondere komplexes, motiviertes Verhalten (ODT) moduliert (Manuskript III) und (d) der PPTg Verhalten wahrscheinlich über die striatalen DA/GLU-Interaktionen beeinflusst (Manuskripte II, III und IV).

Im folgenden Kapitel werden die Ergebnisse der aufgeführten Manuskripte zusammenfassend diskutiert, wobei zuerst die neurochemischen Beziehungen des PPTg mit dem frontostriatalen System und dann die verhaltensrelevanten Befunde betrachtet werden.

5.1. Neuroanatomische Verbindungen des PPTg mit der ventralen Basalganglienschleife

Der PPTg ist anatomisch sowohl direkt als auch indirekt (z.B. Thalamus und limbische Strukturen) mit den Basalganglien verbunden (Hallanger and Wainer, 1988; Steininger et al., 1992; Semba and Fibiger, 1992). Bemerkenswert ist, dass die Vernetzung des PPTg nicht auf die ventrale oder dorsale Basalganglienschleife begrenzt ist. Vielmehr könnte der PPTg als Schnittstelle zwischen limbischen Systemen, der ventralen Basalganglienschleife und senso-motorischen Systemen der dorsalen Schleife fungieren. D.h. auf der Ebene des PPTg wird motivationale Information mit herausgehender motorischer Information abgeglichen (Mogenson, 1987; Winn, 1998; Keating and Winn, 2002). Da verhaltenspharmakologische Daten auf eine Interaktion des PPTg mit der ventralen Basalganglienschleife hindeuteten, wurden die neurochemischen Untersuchungen in der vorliegenden Arbeit auf die Eingangsstruktur der ventralen Schleife (NAC) beschränkt. Aus

diesen Gründen liegt der Schwerpunkt in der folgenden Diskussion auch auf den Interaktionen des PPTg mit der ventralen Basalganglienschleife5.1.1 Neurochemische Interaktionen des PPTg mit frontostriatalen Strukturen

Die Basalganglien sind grundlegend an der Initiierung, Durchführung und Richtung von Verhalten beteiligt. Aus dem Aufbau der Basalganglien (siehe Einleitung) wird deutlich, dass den DA/GLU-Interaktionen, vor allem im NAC, eine Schlüsselfunktion bei der motivationalen Verhaltensmodulation über die Basalganglien zukommt. DA wird dabei als Signal für bewertende und neuigkeitsvermittelnde Information verstanden, welches gleichzeitig auftretende glutamaterge Signale verstärkt oder hemmt. Die glutamatergen Signale aus limbisch-kortikalen Regionen repräsentieren vor allem motivationale Information. Das frontostriatale System (Basalganglien und Afferenzen) im Allgemeinen und die beschriebenen DA/GLU-Interaktionen im Speziellen werden als neuronales Substrat für Verhaltensprozesse (z.B. die *response selection* bei der Verhaltensanpassung) verstanden, die der Ausführung von komplexem, motiviertem Verhalten zugrunde liegen (Salamone et al., 1997; Schmidt, 1998; Koch et al., 2000; Tzschentke and Schmidt, 2000; Horvitz, 2002; Sesack et al., 2003).

Anatomisch ist der PPTg mit frontostriatalen Strukturen verbunden, die an den striatalen DA/GLU-Interaktionen beteiligt sind (Hallanger and Wainer, 1988; Heimer et al., 1991; Steininger et al., 1992; Semba and Fibiger, 1992). Insbesondere stellt der PPTg, neben den kortikalen Regionen, eine weitere gut charakterisierte und prominente Afferenz der dopaminergen Mittelhirnneurone dar (Jackson and Crossman, 1983; Scarnati et al., 1986; Gould et al., 1989; Clements and Grant, 1990; Kalivas, 1993; Kelland et al., 1993; Ford et al., 1995; Scarnati and Florio, 1997; Lokwan et al., 1999). Die Projektion vom VTA zum PPTg scheint dahingegen weniger prominent zu sein. Sie wurde bisher anatomisch und neurochemisch als ’gering markierte’ (*sparsely labeled*) (Steininger et al., 1992; Semba and Fibiger, 1992; Ichinohe et al., 2000) GABAerge Projektion charakterisiert (Laviolette et al., 2002). Allerdings wurden in der vorliegenden

Arbeit, durch neurochemische (Manuskript I) sowie immunhistologische (Anhang) Methoden, weitere dopaminerge Projektionen aus dem VTA zum PPTg nachgewiesen (Manuskript I und Anhang). Darüber hinaus führte eine lokale Stimulation des GABA-Systems (über Baclofen) im PPTg zu einer reduzierten DA-Freisetzung im PPTg (Abbildung 3, Projektion 1) und eine lokale Stimulation des GLU-Systems (über NMDA und AMPA) zu einer verstärkten DA-Freisetzung im PPTg (Abbildung 4, Projektion 1) (Manuskript I). Diese Daten zeigen, dass GABAerge und glutamaterge Information, die wahrscheinlich aus den Basalganglien (z.B. ventrales Pallidum) bzw. limbischen Regionen (z.B. PFC) stammen, mit dopaminerger Information im PPTg interagiert. Der PPTg kann so frontostriatale Information modulieren und an motorische Areale im Hirnstamm und Rückenmark weiterleiten (Winn, 1998). Die dopaminerge Innervation des PPTg deutet darüber hinaus darauf hin, dass der PPTg mehr ist als eine reine Ausgangsstruktur des frontostriatalen Systems, dessen Funktion ausschließlich in der Weiterleitung von Information besteht. Es scheint vielmehr, dass Information die im PPTg bearbeitet wird auch wieder an das frontostriatale System zurückprojiziert wird. Für einen solchen *Feedback*-Mechanismus könnten die Efferenzen zum DA-System von Bedeutung sein. Diese Projektion enthält die exzitatorischen Transmitter Acetylcholin (Gould et al., 1989) und GLU (Scarnati et al., 1986; Clements and Grant, 1990) sowie den inhibitorischen Transmitter GABA (Ford et al., 1995). Die bisherige Charakterisierung dieser Projektion deutet jedoch auf eine größere Bedeutung der exzitatorische Projektion vom PPTg zum VTA hin, denn elektrophysiologische (Kelland et al., 1993; Scarnati and Florio, 1997; Lokwan et al., 1999) sowie pharmakologische (GABA-Antagonist, μ-Rezeptor-Agonist oder Kainat-Rezeptor-Agonist) Stimulationen des PPTg erhöhen die Aktivität dopaminerger VTA-Neurone und führen zu einer erhöhten DA-Freisetzung im NAC (Niijima and Yoshida, 1988; Kalivas, 1993; Klitenick and Kalivas, 1994; Miller et al., 2002). In der vorliegenden Arbeit wurden diese Daten bestätigt und, indem gezeigt wurde, dass auch eine verhaltensinduzierte DA-Freisetzung im NAC von einer pharmakologischen Manipulation des PPTg

abhängig ist, auch erweitert (Manuskript IV). Sowohl eine lokale Stimulationen des GABAergen Systems (über Baclofen) als auch die des glutamatergen Systems (über AMPA) blockierten die verhaltensinduzierte DA-Freisetzung im NAC. Gleichzeitig zu den DA-Effekten induzierte die PPTg-Stimulation auch eine verstärkte GLU-Freisetzung im PPTg. Auffällig ist, dass sowohl eine PPTg-Stimulation (GLU-Agonist) als auch eine PPTg-Inhibition (GABA-Agonist) die mesoaccumbale DA-Freisetzung blocken. Die Tatsache, dass unterschiedliche PPTg-Manipulationen die gleichen DA-Effekte auslösen, lässt darauf schließen, dass das GLU- und das GABA-System unterschiedliche Efferenzen des PPTg, d.h. unterschiedliche Schaltkreise, ansprechen. Während das GABA-System im PPTg wahrscheinlich die oben beschriebene exzitatorische PPTg-Efferenz zu den dopaminergen VTA-Neuronen inhibiert (Abbildung 3, Projektion 2), scheint das GLU-System, zumindest über AMPA-Rezeptoren im PPTg, andere inhibitorische Projektionen zum VTA zu aktivieren:

(a) GABAerge Neurone projizieren vom PPTg zum VTA (Ford et al., 1995). Immunhistologische Daten zeigen, dass 30 – 40 % der anterograd markierten Terminale aus dem PPTg GABA-immunreaktiv sind und auf dopaminergen VTA-Neuronen terminieren (Charara et al., 1996). Eine selektive Stimulation dieser Neurone würde zu einer Inhibition mesoaccumbaler Neurone führen. (Abbildung 4, Projektion 2).

(b) GABAerge Interneurone existieren in dem VTA (Beart and McDonald, 1980; Steffensen et al., 1998) und werden cholinerg (Garzon et al., 1999), wahrscheinlich auch glutamaterg, vom PPTg innerviert (Kalivas et al., 1989; Seutin et al., 1990; Churchill et al., 1992; Wang and French, 1995). Eine selektive exzitatorische Innervation dieser Neurone würde zu einer Inhibition mesoaccumbaler Neurone führen (Abbildung 4, Projektion 3).

(c) Der zusätzlich gemessene GLU-Anstieg im PPTg deutet auf *long-loop*-Verbindungen hin, die z.B. den PFC mit einbeziehen (Abbildung 4, Projektion 4). Dopaminerge VTA-Neurone, die cholinerg (wahrscheinlich auch glutamaterg) vom PPTg innerviert werden, weisen eine charakteristische DA-Transporter-Expression

auf, die auf mesokortikale aber nicht mesoaccumbale Neurone hindeuten (Garzon et al., 1999). Eine selektive Stimulation dieser Neurone würde zu einer erhöhten DA-Freisetzung im PFC führen. Übereinstimmend mit den Daten der vorliegenden Arbeit zeigen Verhaltensstudien und neurochemische Studien, dass die DA-Freisetzung im PFC sowohl eine Verhaltensaktivierung als auch eine DA-Freisetzung im NAC hemmt. Die beteiligten Projektionsbahnen sind zwar nicht genau beschrieben, es werden aber vor allem zwei Bahnen diskutiert: (a) Eine glutamaterge Rückprojektion vom PFC auf PPTg-Neurone, die wiederum direkt (Projektion 2, Abbildung 4) oder indirekt (Projektion 3, Abbildung 4) inhibitorisch die mesoaccumbalen DA-Neurone innervieren und (b) eine glutamaterge Projektion zu GABAergen NAC-Neuronen, die die mesoaccumbalen DA-Neurone innervieren (als Übersicht siehe Tzschentke and Schmidt, 2000; Sesack et al., 2003). Eine PPTg-Stimulation würde demnach die DA-Freisetzung z.B. im PFC erhöhen und die glutamatergen PFC-PPTg-Neurone und/oder PFC-NAC-Neurone aktivieren. Dies würde im Endeffekt zu einer Reduzierung des accumbalen DA führen. Bestätigt wird ein solcher Schaltkreis durch Daten die zeigen, dass die meisten DA-Rezeptoren im PFC exzitatorisch wirkende D_1/D_5-Rezeptoren sind (Smiley et al., 1994; Bergson et al., 1995). Des Weiteren bestätigen elektrophysiologische und pharmakologische Studien eine D_1-Rezeptor-induzierte Beeinflussung glutamaterger PFC-Neurone (Geijo-Barrientos and Pastore, 1995; Yang and Seamans, 1996; Goldman-Rakic et al., 2000; Henze et al., 2000; Seamans et al., 2001). Es gibt aber auch Studien, die auf eine inhibitorische Wirkung von DA auf PFC-Neurone hinweisen (Bunney and Aghajanian, 1976; Mora et al., 1976; Mantz et al., 1988). In der vorliegenden Arbeit kann nicht geklärt werden, welche Wirkung DA im PFC ausübt. Die Existenz von Subpopulationen, z.B. in PFC-VTA-Projektionsneuronen, lässt aber vermuten, dass es eine spezifische DA-Rezeptorverteilung gibt (Carr and Sesack, 1999; Sesack and Carr, 2002). Die in der vorliegenden Arbeit gemessene gesteigerte GLU-Freisetzung im PPTg sowie die reduzierte DA-Freisetzung im NAC nach PPTg-Stimulation

(AMPA) deuten darauf hin, dass zumindest auf den PFC-PPTg- und PFC-NAC-Neuronen exzitatorische DA-Rezeptoren expremiert sind.

Die neurochemischen Daten der vorliegenden Arbeit zeigen, dass das GABA- sowie das GLU-System auf der Ebene des PPTg mit dem dopaminergen System interagieren und damit die Informationsverarbeitung im frontostriatalen System, speziell im VTA und NAC, beeinflusst. Über welche genauen anatomischen Projektionen diese Interaktionen umgesetzt werden, kann im Rahmen dieser Arbeit nicht hinreichend beantwortet werden. Während für das GABA-System im PPTg direkte Verbindungen zur VTA wahrscheinlich sind, deuten die DA- und GLU-Effekte nach Stimulation des GLU-Systems im PPTg aber auf *long-loop*-Verbindungen hin, die z.B. den PFC mit einschließen.

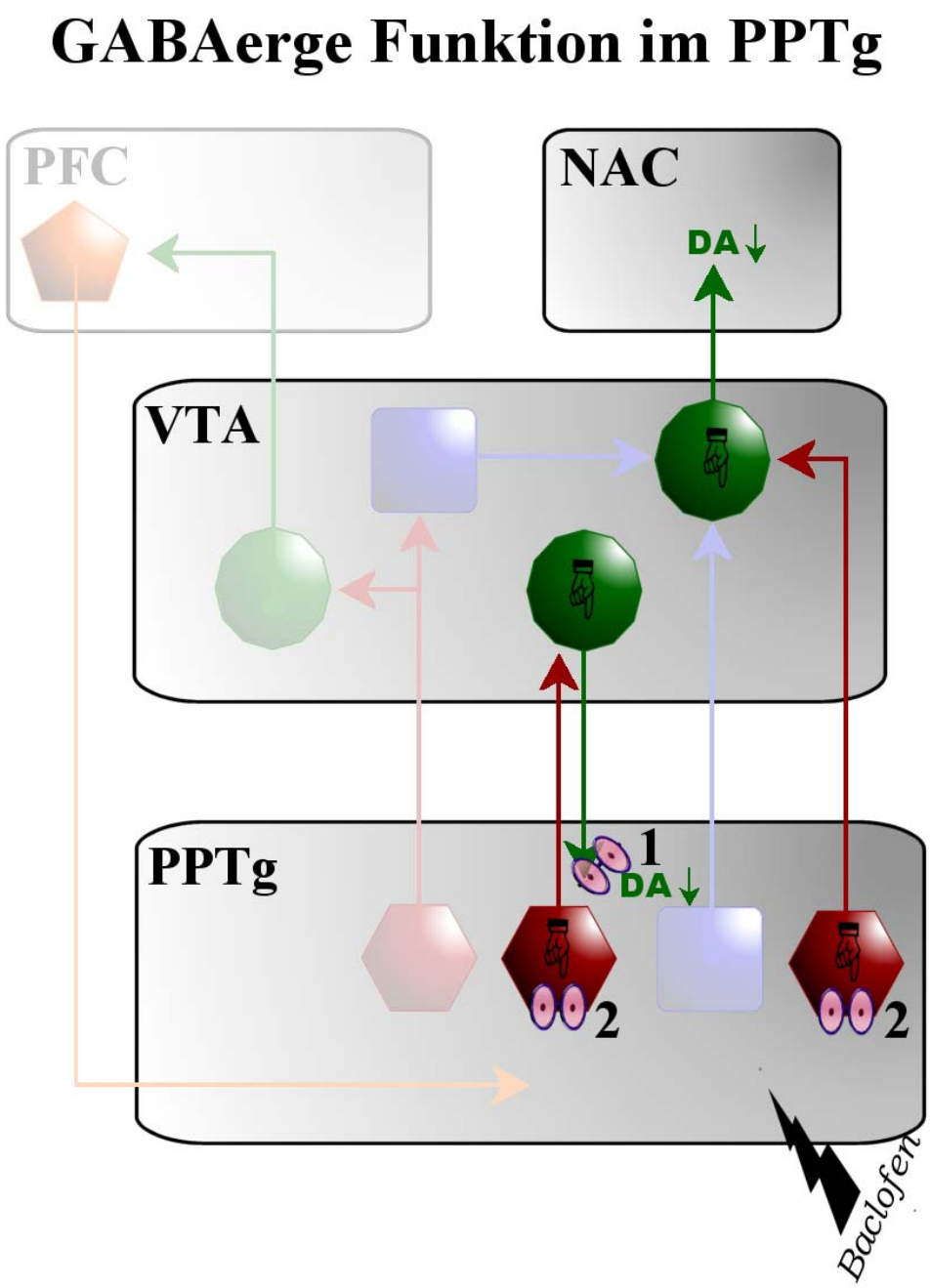

Abbildung 3: Projektionsschaltkreise, die den neurochemischen Effekten nach Baclofen-Administration in den PPTg zugrunde liegen. Rote Sechsecke = glutamaterge/cholinerge Neurone, blaue Vierecke = GABAerge Neurone, grüne Kreise = dopaminerge Neurone, orangene Fünfecke = glutamaterge Neurone, = Baclofen Administration, transparente Strukturen entsprechen Strukturen die zusätzlich die AMPA-induzierten Effekte vermitteln, = $GABA_B$-Rezeptor, schwarze Hand = reduzierte Aktivität, kleine DA/GLU-Pfeile markieren gemessene Änderungen in der Transmitterfreisetzung. Die Nummern 1 und 2 beziehen sich auf Projektionsbahnen im Text.

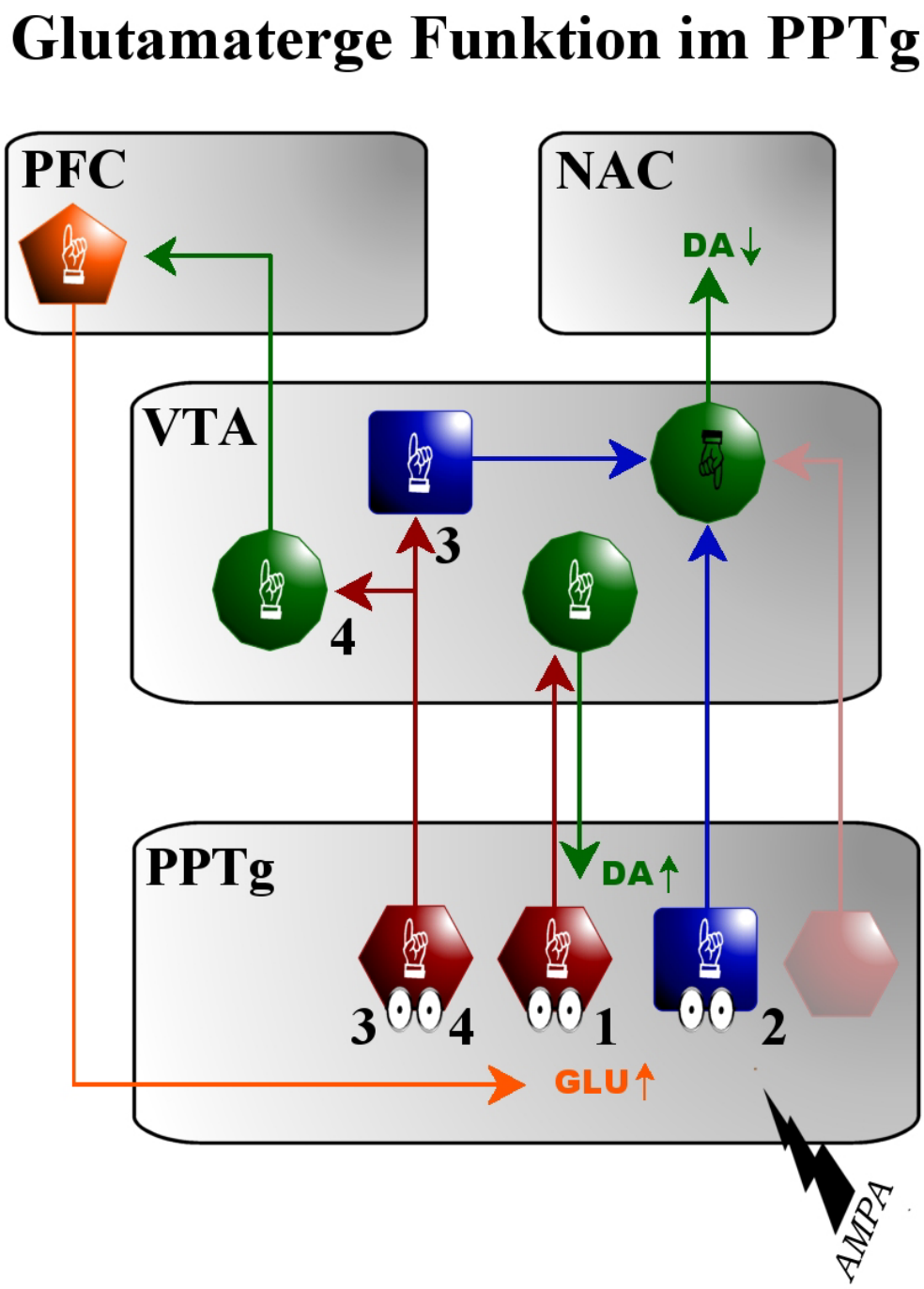

Abbildung 4: Projektionsschaltkreise, die den neurochemischen Effekten nach AMPA-Administration in den PPTg zugrunde liegen. Rote Sechsecke = glutamaterge/cholinerge Neurone, blaue Vierecke = GABAerge Neurone, grüne Kreise = dopaminerge Neurone, orangene Fünfecke = glutamaterge Neurone, ↯ = AMPA Administration, 👀 = AMPA-Rezeptor, weiße Hand = erhöhte Aktivität, schwarze Hand = reduzierte Aktivität, kleine DA/GLU-Pfeile markieren gemessene Änderungen in der Transmitterfreisetzung. Die Nummern 1 – 4 beziehen sich auf die Projektionsbahnen im Text.

5.2. Verhaltensrelevante Funktionen des PPTg

Wie oben bereits erwähnt werden die DA/GLU-Interaktionen im frontostriatalen System, vor allem im NAC, als ein zentrales Element von Verhaltensanpassungsprozessen angesehen. DA wird dabei als Signal für bewertende und neuigkeitsvermittelnde Information verstanden, welches gleichzeitig auftretende glutamaterge Signale, die motivationale Information vermitteln, verstärkt oder hemmt. Dieser neurochemische Mechanismus wird als direktes neuronales Substrat der *response selection* angesehen und stellt somit einen grundlegenden Mechanismus der motivationalen Verhaltensanpassung dar (Salamone et al., 1997; Schmidt, 1998; Koch et al., 2000; Tzschentke and Schmidt, 2000; Horvitz, 2002; Sesack et al., 2003).

Die Bedeutung des PPTg bei einfachem, sehr grundlegendem Verhalten, wie z.B. der Motorik und dem Schlaf-Wach-Zyklus ist gut etabliert. Zudem deuten die oben beschriebenen anatomischen Verbindungen (siehe 5.1.) sowie die in dieser Arbeit nachgewiesenen neurochemischen Interaktionen (siehe 5.1.1.) des PPTg mit den frontostriatalen Strukturen auf eine weitere Funktion des PPTg in komplexem Verhalten, wie z.B. kognitives oder motiviertes Verhalten, hin.

In Übereinstimmung mit den anatomischen und neurochemischen Daten, bestätigen die Verhaltensuntersuchungen der vorliegenden Arbeit eine Funktion des PPTg in komplex-motiviertem Verhalten. PPTg-Manipulationen (Läsion, Inhibition über das GABA-System sowie Stimulation über das AMPA-System) beeinflussten spezifisch die Ausführung einer komplexen Verhaltensaufgabe, hier die Ausführung des ODT, bei der die Tiere durch eine Futterbelohnung motiviert wurden, war beeintraechtigt (Manuskript III, IV). Einfacheres, motorisch-motiviertes Verhalten (hier die Exploration), welches nur bedingt höhere kognitive Anforderungen stellt und ausschliesslich natürliches Verhalten (z.B. Lokomotion, Aufrichten) abruft (im Gegensatz zu operanten Verhaltensaufgaben, bei denen ein Verhalten erst erlernt wird (Hebeldruecken)), wurde dahingegen nur dann durch PPTg-Manipulationen beeinflusst, wenn gleichzeitig entweder das dopaminerge

(über Amphetamin) oder das glutamaterge System (über MK-801) stimuliert war (Manuskript II). Initiation von motorischem Verhalten konnte über PPTg-Manipulationen dahingegen nicht ausgelöst werden (Manuskript I). Andere Authoren haben gezeigt, dass dies selbst dann nicht der Fall, wenn gleichzeitig das dopaminerge System stimuliert wird (Olmstead and Franklin, 1994; Inglis et al., 1994b). Diese Daten zeigen, dass durch PPTg-Manipulationen ausgelöste Verhaltensänderungen vom motivationalen Status des Tieres und von der Komplexität des modulierten Verhaltens abhängen. Diese Kausalität wird von Studien bestätigt, die ebenfalls Zusammenhänge zwischen PPTg-induzierten Effekten und komplexem Verhalten sowie Motivationsstatus der Tiere gefunden haben: PPTg-lädierte Tiere waren bei der Ausführung von räumlichen Gedächtnisaufgaben nur beeinträchtigt, wenn die Tiere unter verhältnismäßig vielen Auswahlmöglichkeiten entscheiden mussten, nicht aber wenn es nur wenige Auswahlmöglichkeiten gab: z.B. das Erinnern der Position eines belohnten Arms in einem 8-Arm-Labyrinth gegenüber der Position in einem 4-Arm-Labyrith oder das Erreichen einer nicht-sichtbaren Plattform gegenüber einer sichtbaren Plattform in einem Wasserlabyrinth (Dellu et al., 1991). Die PPTg-läsionsinduzierte Blockade einer Futter-, Opioid- oder Amphetamin- konditionierten Platzpräferenz war dahingegen abhängig vom Motivationsstatus des Tieres, d.h. es war abhängig davon, ob die Tiere futterdepriviert bzw. substanzabhängig oder satt bzw. substanznaiv waren (Bechara and van-der-Kooy, 1989; Olmstead and Franklin, 1994; Bechara et al., 1998). Auch in anderen verhaltenspharmakologischen Studien wurden bereits die Auswirkungen einer PPTg-Läsion auf komplex-motiviertes Verhalten untersucht (als Übersicht siehe Steckler et al., 1994; Winn, 1998; Steiniger, 1999). Diese Studien zeigen, eine Beeinträchtigung von PPTg-lädierten Tieren bei der Ausführung von Verhaltensmodellen, wie z.B. konditionierte Platzpräferenz, intra-kraniale Selbststimulation, konditioniertes operantes Antwortverhalten und Lern- bzw. Gedächtnisaufgaben. Kontrovers diskutiert werden jedoch die Mechanismen über die der intakte PPTg komplexes, motiviertes Verhalten beeinflusst.

Wie oben bereits angesprochen machen die anatomischen Verbindungen (siehe 5.1.) sowie die in dieser Arbeit nachgewiesenen neurochemischen Interaktionen (siehe 5.1.1) des PPTg mit den frontostriatalen Strukturen eine Beteiligung des PPTg bei motivationalen Verhaltenseffekten wahrscheinlich. Da das frontostriatale System maßgeblich für die Verhaltensanpassung, vor allem über die Verarbeitung der *response selection*, verantwortlich ist, liegt es nahe, dass auch der PPTg die *response selection* beeinflusst. Auch der oben aufgeführte Zusammenhang, nach dem eine hohe Motivation des Tieres sowie eine hohe Komplexität des Verhaltens die Bedeutung des PPTg für das auszuführenden Verhalten erhöht, deutet auf die Beteiligung des PPTg in frontostriatalen Mechanismen hin: Erstens ist dieses System für die Verarbeitung von motivationaler Information von großer Bedeutung (Mogenson, 1987; Pennartz et al., 1994; Groenewegen et al., 1996; Groenewegen et al., 1997) und zweitens resultiert eine erhöhte Komplexität der Verhaltensaufgaben in erhöhten Anforderungen an die Verhaltensanpassung der Tiere (Salamone et al., 1997; Schmidt, 1998; Redgrave et al., 1999; Koch et al., 2000; Gurney et al., 2001; Horvitz, 2002; Sesack et al., 2003). Dieser Zusammenhang wird insbesondere in dem in dieser Arbeit entwickeltem ODT verdeutlicht, denn das beständige, aber zufällige Wechseln der Positionen von belohnten und unbelohnten Tasten, in diesem Test, fordert eine kontinuierliche Anpassung des Verhaltens der Tiere. PPTg-Läsionen sowie -Inhibitionen führten in diesem Test zur drastisch reduzierten *response*-Genauigkeit. Ein solches Defizit deutet auf eine beeinträchtigte *response selection* hin (Manuskript III und IV). Auch die synergistische Wirkung zwischen einer PPTg-Läsion und einer Stimulation des DA-Systems, bzw. Inhibition des GLU-Systems auf die Ausführung von motiviertem Verhalten (Manuskript II und III), unterstützen die Hypothese einer Beteiligung des frontostriatalen Systems an den PPTg-abhängigen Verhaltenseffekten, da DA und GLU zentrale Transmitter im frontostriatalen System sind. Auch die in Manuskript IV gefundene Korrelation zwischen PPTg-Manipulation, DA-Freisetzung im NAC, GLU-Freisetzung im PPTg und der Ausführung des ODT deutet eine solche funktionelle Verbindung an. Diese Daten

zeigen, dass eine hohe *response*-Rate eine hohen DA-Freisetzung im NAC auslöst, während eine geringe *response*-Rate, ausgelöst durch PPTg-Manipulationen (Stimulation über AMPA und Inhibition über Baclofen), die DA-Freisetzung hemmt und im Falle einer PPTg-Stimulation die GLU-Freisetzung im PPTg verstärkt. Dies könnte ein Kompensationssignal für die *response*-Genauigkeit darstellen, da interessanterweise nur nach einer PPTg-Inhibition die *response*-Genauigkeit reduziert war, dies jedoch nicht zu einer erhöhten GLU-Freisetzung im PPTg führte (siehe auch 5.1.1.). Dahingegen führte die PPTg-Stimulation zu einer GLU-Freisetzung im PPTg, jedoch nicht zu einer reduzierten *response*-Genauigkeit.

Bestätigt werden diese Daten auch durch Studien die zeigen, dass PPTg-lädierte Tiere bei der korrekten Ausführung von operanten (Inglis et al., 1994b; Inglis et al., 2000; Alderson et al., 2002) sowie nicht-operanten Diskriminierungsaufgaben beeinträchtigt sind (Olmstead et al., 1999; Inglis et al., 2001). Zudem führen Läsionen im frontostriatalen System (NAC, PFC) und im PPTg zu vergleichbaren Verhaltensdefiziten: NAC-Läsionen führen in operanten Aufgaben, zu verminderter *response*-Rate und –Genauigkeit, entsprechend der in Manuskript III beschriebenen Effekte nach PPTg-Läsionen (Balleine and Killcross, 1994). Ebenso wurden nach PPTg- wie auch PFC-Läsionen Defizite in der Genauigkeit bei einer Stimulus-Diskriminierung (*discriminative accuracy*) in der *five-serial-reaction-time*-Aufgabe beschrieben (Muir et al., 1996; Inglis et al., 2001). Auch in den Gedächtnistests *random-foraging*- und *delayed-spatial-win-shift*, in denen die Leistung des räumlichen und des Kurzzeitgedächtnisses untersucht werden, wurden nach Inaktivierung des PFC, NAC oder PPTg Verhaltensdefizite beschrieben, die auf eine Beeinträchtigung der *response selection* hindeuten (Floresco et al., 1997; Floresco et al., 1999; Keating and Winn, 2002).

Aus den oben beschriebenen Daten kann daher abgeleitet werden, dass einer PPTg-induzierten Beeinflussung von komplex-motiviertem Verhalten, auf der neurochemischen Ebene eine Modulation der DA/GLU-Interaktion im

frontostriatalen System und auf der Verhaltensebene eine Modulation der *response selection* zugrunde liegt.

Die funktionelle Verbindung des PPTg zum frontostriatalen System könnte neben der Modulation der *response selection* (siehe oben) auch auf motorische Mechanismen hindeuten, über die der PPTg komplex-motiviertes Verhalten beeinflusst. In diesem Zusammenhang hat Mogenson eine 'Achse' vom NAC über das ventrale Pallidum zum PPTg beschrieben über die lokomotorische Information vom NAC an motorische Systeme im Rückenmark weitergeleitet wird (Mogenson, 1987; Mogenson et al., 1993). Neuere Daten deuten jedoch an, dass NAC vermittelte Lokomotion, nicht über den PPTg (Swerdlow and Koob, 1987; Inglis et al., 1994b), sondern über den mediodorsalen Thalamus weitergeleitet wird (Swerdlow and Koob, 1987). Darüber hinaus zeigen 'Greif-Tests' (*grasping task*), dass auch die Feinmotorik, die in operanten Verhaltenstests von größerer Bedeutung ist als die Lokomotion, nicht durch PPTg-Läsionen beeinträchtigt wird (Dunbar et al., 1992). Auch operantes Antwortverhalten auf einen konditionierten Verstärker zeigte erst bei hohen Drück-Belohnungs-Verhältnissen in einem *progressive ratio schedule* ein PPTg-läsionsinduziertes Defizit in der *response*-Rate. Die Ausführung von Tastendrücken per se scheint demnach nicht beeinträchtigt zu sein (Alderson et al., 2002). Zudem sind PPTg-läsionsinduzierte Defizite auch in nicht-operanten Diskriminierungsaufgaben, also in Aufgaben in denen die Feinmotorik keine Rolle spielt, beschrieben (Olmstead et al., 1999; Inglis et al., 2001). Diese Daten sprechen gegen einen rein motorischen Mechanismus über den der PPTg komplex-motiviertes Verhalten beeinflusst.

Die funktionelle Verbindung des PPTg zum frontostriatalen System könnte auch auf Belohnungsmechanismen hindeuten, über die der PPTg komplex-motiviertes Verhalten beeinflusst. Vor allem das mesoaccumbale DA-System wird seit langem als zentrales Element des so genannten 'Belohnungssystems' angesehen (Wise, 1978). Es wird jedoch zunehmend deutlicher, dass auch andere Strukturen (z.B. der PFC) und damit auch andere Transmitter (z.B. GLU), an der Vermittlung belohnender Wirkungen von natürlichen 'Verstärkern' (z.B. Nahrung) aber auch

von Suchtmitteln beteiligt sind (Schmidt and Kretschmer, 1997; Bardo, 1998; Tzschentke and Schmidt, 2003). An dieser Stelle soll angemerkt werden, dass früher die DA-Freisetzung im NAC als 'Belohnungssignal' angesehen wurde, welches eventuell die Stärke einer Belohnung kodiert. Mittlerweile wird eher davon ausgegangen, dass DA die biologische oder motivationale Signifikanz eines Reizes vermittelt (Di Chiara, 1995), und damit, wie oben bereits erwähnt, eher eine bewertende Funktion hat (Salamone et al., 1997; Schmidt, 1998; Koch et al., 2000; Horvitz, 2002; Sesack et al., 2003). Hieraus wird ersichtlich, dass die oben beschriebene Funktion des frontostriatalen Systems in der Verhaltensanpassung und die hier beschriebene Funktion als Belohnungssystem sich nicht widersprechen, sondern als komplementär anzusehen sind.

Es ist in jedem Fall möglich, dass die PPTg-läsionsinduzierte Beeinträchtigung der Durchführung einer futterbelohnten Verhaltensaufgabe (z.B. ODT) aus einer Beeinflussung des Belohnungsmechanismus des frontostriatalen Systems resultiert. Tatsächlich wurden in einigen Studien die Verhaltensveränderungen nach PPTg-Modulation auf eine verminderte Belohnungswahrnehmung zurückgeführt: In einer *progressive-ratio-schedule*-Aufgabe, verzichteten PPTg-lädierte Tiere früher als Kontrolltiere darauf einen Hebel für eine intravenöse Heroininjektionen zu drücken (reduzierter *break point*) (Olmstead et al., 1998). PPTg-Läsion oder -Inhibition reduzierte die *response*-Rate für eine belohnend wirkenden intra-kraniellen Selbststimulation (Yeomans et al., 1993; Lepore and Franklin, 1996), und eine konditionierte Platzpräferenz auf belohnend wirkende Stimuli, wie Amphetamine, Heroin oder Futter wird durch eine PPTg-Läsion geblockt (Olmstead and Franklin, 1994; Bechara et al., 1998). Neuere Studien widersprechen jedoch dem Konzept einer PPTg-abhängigen veränderten Belohnungswahrnehmung: In Tests bei denen die Einnahme, Wahl und Unterscheidung von unterschiedlich dosierten Zuckerlösungen (niedrige Zuckerlösung entspricht einer geringeren Belohnung als eine hohe Zuckerlösung) untersucht wurden, reagierten PPTg-lädierte und Kontrolltiere gleichermaßen auf unterschiedliche Belohnungensstärke (Olmstead et al., 1999; Alderson et al., 2001; Keating et al., 2002). Darüber hinaus sind PPTg-

lädierte Tiere in der Ausführung von operanten Aufgaben mit geringem Drück/Belohnungs-Verhältnis (*low schedule requirements*) nicht beeinträchtigt: Beide Experimentgruppen sind gleich schnell beim Einsammeln der Futterbelohnung in dieser Verhaltensaufgabe, (Alderson et al., 2002) sowie in 8-Arm-Labyrinth-Aufgaben (Keating and Winn, 2002). Diese Daten deuten auf eine 'normale', d.h. unveränderte Belohnungswahrnehmung von PPTg-lädierten Tieren hin.

Die verhaltenspharmakologischen Daten dieser Arbeit zeigen, dass eine hohe Motivation des Tieres sowie eine hohe Komplexität des Verhaltens die Bedeutung des PPTg für das auszuführende Verhalten erhöht. Dieser Zusammenhang, die durch PPTg-Manipulationen induzierten Verhaltensdefizite vor allem in der *response*-Genauigkeit sowie die verhaltensrelevanten Interaktionen einer PPTg-Manipulation mit dem dopaminergen und glutamatergen System deuten auf frontostriatale Mechanismen hin. Dabei scheint der PPTg komplex-motiviertes Verhalten durch eine Modulation der DA/GLU-Interaktion im frontostriatalen System zu beeinflussen, indem speziell die *response selection* beeinflusst wird.

5.3. Zusammenfassung der neurochemischen und verhaltensrelevanten Diskussion

Die Bedeutung des PPTg bei einfachem, sehr grundlegendem Verhalten, wie z.B. der Motorik und dem Schlaf-Wach-Zyklus ist gut etabliert. In der vorliegenden Arbeit wurde darüber hinaus gezeigt, dass eine hohe Motivation des Tieres sowie eine hohe Komplexität des Verhaltens die Bedeutung des PPTg für das auszuführende Verhalten erhöht. Der PPTg ist demnach an der Verarbeitung von komplexem, motiviertem Verhalten beteiligt, wahrscheinlich über eine Beeinflussung der *response selection*. Dieser Befund deutet auf eine Interaktion des PPTg mit dem frontostriatalen System hin, da in diesem System die motivationale Information aus limbisch-kortikalen Regionen, mit dem motorischen Ausgang der Basalganglien integriert wird. Hierüber wird die motivationale Verhaltensanpassung entscheidend beeinflusst, wobei diesem Prozess vor allem

DA/GLU-Interaktionen im frontostriatalen System zugrunde liegen, über die direkt die *response selection* verarbeitet wird. Die in dieser Arbeit gezeigte neurochemische Interaktion des PPTg mit dem DA-System im NAC sowie dem GLU-System, wahrscheinlich aus dem PFC unterstützt dieses Konzept. Darüber hinaus wurde gezeigt, dass die Position des PPTg zum frontostriatalen System nicht nur als Ausgangsstruktur betrachtet werden darf. Der PPTg scheint vielmehr eine *long-loop*-Verbindung darzustellen, über die verhaltensrelevante Information zurück ins frontostriatale System projiziert wird. Diese Position gibt dem PPTg die Voraussetzung die DA/GLU-Interaktionen im frontostriatalen System und somit die *response selection* zu modulieren, wie es aufgrund der Verhaltensstudien postuliert wird.

6. Anhang

6.1. Immunhistochemische Studie zur Untersuchung dopaminerger Innervation des PPTg

Der PPTg stellt, neben den kortikalen Regionen, eine weitere gut charakterisierte und prominente Afferenz der dopaminergen Mittelhirnneurone VTA und SNc dar (Jackson and Crossman, 1983; Scarnati et al., 1986; Gould et al., 1989; Clements and Grant, 1990; Kalivas, 1993; Kelland et al., 1993; Ford et al., 1995; Scarnati and Florio, 1997; Lokwan et al., 1999). Die umgekehrte Projektion aus dem VTA und der SNc zum PPTg scheint dahingegen weniger prominent zu sein (Steininger et al., 1992; Semba and Fibiger, 1992; Ichinohe et al., 2000). Obwohl bisher erst GABA als Transmitter dieser Projektion charakterisiert wurde (Laviolette et al., 2002), wurde in Manuskript I gezeigt, dass DA im PPTg dialysiert werden kann. Die Daten dieser Arbeit deuten auf synaptisch freigesetztes DA hin, allerdings kann eine Diffusion von somatodendritisch freigesetztem DA aus dem VTA, der SNc aber auch dem retrorubralen Nucleus zum PPTg nicht ausgeschlossen werden. Deshalb wurde in der vorliegenden Arbeit über immunhistochemische Methoden die Existenz einer dopaminergen Afferenz des PPTg untersucht.

In dieser Studie wurde über standardisierte stereotaktische Methoden der Farbstoff Fluorogold in den PPTg infundiert. Fluorogold wird über Terminale in Neurone aufgenommen und nach retrogradem, axonalem Transport im Zellkörper dieser Neurone abgelagert. Somit werden über Fluorogold, afferente Neurone der Injektionsregion markiert. Nach der Entnahme der Gehirne wurden immunhistologische Methoden (siehe unten) angewandt, um die Tyrosinhydroxylase (TH) in den Gehirnschnitten zu markieren. Tyrosinhydroxylase ist ein Enzym, das am Syntheseweg der Katecholamine, d.h. DA, Noradrenalin und Adrenalin, beteiligt ist. Doppeltmarkierte Zellen, sind demnach alle afferenten katecholaminergen Zellen des PPTg. Der Nachweis

doppeltmarkierter Zellen in den dopaminergen Nuclei, SNc und VTA, ist der immunhistochemische Beweis für eine dopaminerge Innervation des PPTg.

Es wurden doppeltmarkierte Zellen im Bereich der SNc (Abbildung 4) und dem VTA gefunden. Zusammenfassend bestätigt dieser Befund die Mikrodialyse Daten des Manuskript I, wonach im PPTg dialysiertes DA aus afferenten Neuronen des PPTg freigesetzt werden. Auffällig ist, dass neben TH positiven SNc- und VTA-Neuronen auch nicht-TH-positive Neurone in dem VTA und der SNc gibt, die zum PPTg projizieren. Somit bestätigt diese Arbeit neben den dopaminergen (Manuskript I), auch die nicht-dopaminergen (wahrscheinlich GABAerge) (Laviolette et al., 2002) Projektionen vom VTA und der SNc zum PPTg.

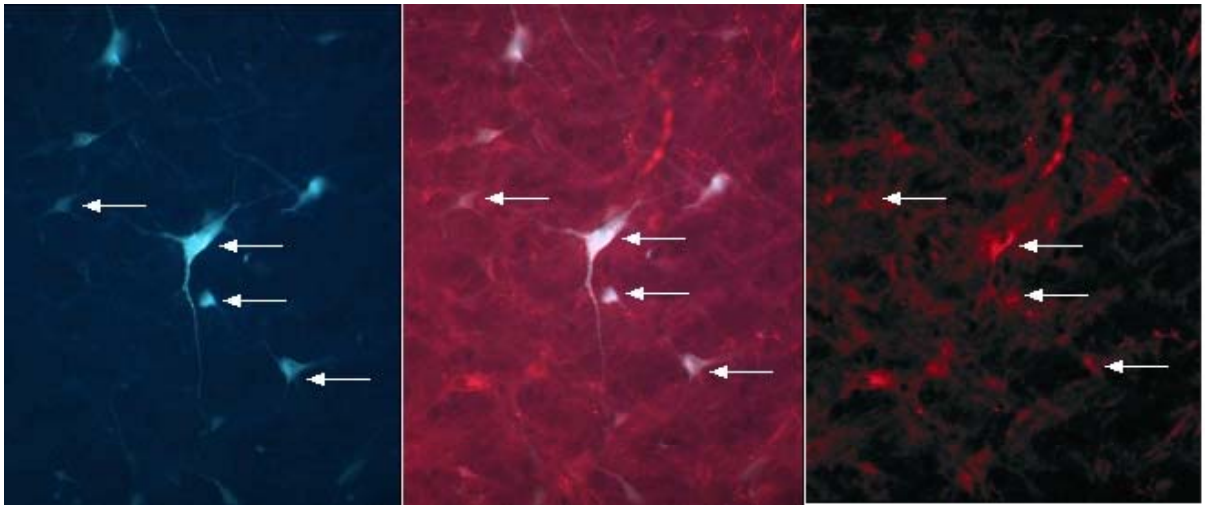

Abbildung 4 Immunhistochemische Studie zur Untersuchung dopaminerger Innervation des PPTg. (A) fluorogold-markierte Zellen, (B) doppeltmarkierte Zellen, (C) Tyrosinhydroxilase-markierte Zellen. Die Pfeile markieren die selben Zellen in allen drei Aufnahmen. Die Aufnahmen zeigen einen Ausschnitt der SNc.

7. Literaturverzeichnis

Aufgeführt sind alle Literaturquellen, auf die ich außerhalb der publizierten oder zur Publikation eingereichten Manuskripte verweise. Literaturhinweise innerhalb der Manuskripte wurden am Ende des jeweiligen Manuskripts aufgeführt.

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8. Abkürzungen

AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolpropionsäure
ARAS	aufsteigendes aktivierendes System
DA	Dopamin
GABA	γ-Amino-Buttersäure
GLU	Glutamat
HPLC	Hochdruckflüssigkeitschromatographie
MK-801	(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imin
NAC	Nucleus accumbens
ODT	operanter Diskriminierungstest
PFC	präfrontaler Kortex
PPTg	pedunculopontiner tegmentaler Nucleus
SNc	Substantia nigra pars compacta
STN	subthalamischer Nucleus
TH	Tyrosinhydroxilase
VTA	ventrales tegmentales Areal

9. Erklärung zum Eigenanteil an den vorgelegten Manuskripten

Mein Eigenanteil an den in dieser Doktorarbeit aufgeführten Manuskripten (I – IV) umfasste die gesamte konzeptionelle und inhaltliche Planung, sowie die Vorbereitung, Durchführung und Auswertung der Versuche und die Verfassung der Manuskripte.

Zu keiner Zeit ging der Anteil von Beate D. Kretschmer über das im Rahmen eines Betreuungsverhältnis übliche Maß hinaus.

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