Published in final edited form as:

Psychopharmacology (Berl). 2014 December ; 231(24): 4695–4701. doi:10.1007/s00213-014-3620-4.

Differential effects of amphetamine and GBR-12909 on orolingual motor function in young vs aged F344/BN rats

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Abstract

Rationale—Orolingual motor deficits, such as dysarthria and dysphagia, contribute to increased morbidity and mortality in the elderly. In preclinical studies we and others have reported age-related decreases in tongue motility in both F344 and F344/BN rats. The fact that these deficits are associated with nigrostriatal dopamine (DA) tissue measures suggests that increasing dopamine function might normalize tongue motility.

Objective—The purpose of the current study was to determine whether two indirect dopamine agonists with locomotor-enhancing effects, d-amphetamine (amphetamine; 1 & 2 mg/kg) and GBR-12909 (5, 10, and 20 mg/kg), can improve tongue motility in aged F344/BN rats.

Methods—Young (6 months) and aged (30 months) F344/BN rats licked water from an isometric force disc so that tongue motility (licks/second) and tongue force could be measured as a function of age and drug dose.

Results—Consistent with our previous studies, tongue force was greater and tongue motility was lower in the aged group. Tongue motility was increased by amphetamine but not by GBR-12909. Amphetamine decreased peak tongue force, primarily in the young group. GBR-12909 did not affect tongue force. GBR-12909 increased the number of licks/session in the young group but not in the aged group, while amphetamine increased this measure in both groups.

Conclusion—These results demonstrate differential effects of these drugs on orolingual motor function and suggest that blocking DA uptake is insufficient to increase tongue motility in aging.

Keywords

aging; aging; oromotor; bradykinesia; movement; licking; operant; tongue

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1. Introduction

Normal aging is accompanied by decreases in quantity and quality of motor activity in humans and animals (Bennett et al., 1996; Ingram, 2000). In addition to locomotor and gait deficits, diminished orolingual motor function is associated with human aging. These alterations contribute to dysarthria (deficits in speech articulation), dysphagia (swallowing deficits) and masticatory (chewing) deficits. The consequences of these deficits range from increased social isolation (in the case of dysarthria; Clark, 1994), to increased mortality and morbidity (in the case of dysphagia; Baum and Bodner, 1983; Fucile et al., 1998; Lieu et al., 2001). While orolingual deficits have been studied extensively in elderly human volunteers, very few studies have examined age-related changes in orolingual motor function using animal models. Preclinical studies using animal models are critical to determining the neural mechanisms that underlie normal and abnormal motor function. They are also essential to testing interventions for motor deficits in aging and disease (LeDoux, 2005).

In clinical studies, tongue protrusion force and motility are often used as indices of orolingual motor function (e.g., Crow and Ship, 1996; Fei et al., 2013; Hirai et al., 1991). We and others have reported tongue motility (but not force) deficits in aged F344 (Stanford et al., 2003; Zhang et al., 2008) and F344/BN (Nagai et al., 2008; Zhang and Stanford, 2008; Zhang et al., 2008) rats. In these studies, aged rats that are trained to lick water from a force-sensing surface exhibited slower licking speed (licks/second) than their younger counterparts. Tongue motility was positively correlated with nigrostriatal tissue levels of dopamine (DA) and DA turnover (Stanford et al., 2003). Because nomifensine, a mixed DA/norepinephrine (NE) uptake inhibitor, increases locomotor activity in aged rats (Hebert and Gerhardt, 1998; Stanford et al., 2002a, 2002b), we hypothesized that it would improve tongue motility in aged rats. Our findings did not support our hypothesis, however (Stanford et al., 2003), and show that merely blocking uptake of DA (and/or NE) is not sufficient to increase tongue motility in aged rats.

The purpose of the current study was to extend our previous findings by determining whether tongue motility deficits could be reversed by a drug that increases DA neurotransmission: d-amphetamine (amphetamine), and by a drug that is more selective for the DA transporter: GBR-12909. Amphetamine enhances extracellular DA levels by reversing DA transport (i.e., releasing DA) through the DA transporter. Amphetamine also increases serotonin (5-HT) and NE levels (Kuczenski et al., 1995; Segal and Kuczenski, 1997), likely through a similar mechanism of action involving the 5-HT and NE transporters. Like amphetamine and nomifensine, the DA uptake inhibitor GBR-12909 increases locomotor activity in rats (Kelley and Lang, 1989; van den Buuse and de Jong, 1989). Unlike amphetamine and nomifensine, GBR-12909 is highly selective for the DA transporter (Andersen, 1989), increasing extracellular DA levels (Budygin et al., 1997; Camerero et al., 2002) by blocking DA uptake. GBR-12909 has not been found to reverse DA transport. Our results with nomifensine suggest that merely blocking DA uptake is insufficient to improve tongue motility in aged rats. The hypothesis of the current study was that because of its DA- (and perhaps NE- and 5-HT-) releasing properties, amphetamine would increase tongue motility in the aged group, while GBR-12909 would not.

2. Materials and methods

2.1. Animals

Young (6-month-old) and aged (30-month-old) male F344/BN rats (n=10/age group) were obtained from the National Institute on Aging colonies (Harlan Sprague-Dawley, Indianapolis, IN). Procedures were approved by the University of Kansas Medical Center IACUC and adhered to the *Guide for the Care and Use of Laboratory Animals* (1996).

2.2. Experimental chambers

We used the experimental chamber that was designed and first used by Fowler et al. (Fowler and Das, 1994; Fowler and Mortell, 1992) and in our recent studies (Smittkamp et al., 2008, 2010; Stanford et al., 2003). Data were recorded in a modified rodent operant chamber with a front panel containing a 6-cm square hole at floor level. Affixed to the square hole was a 6-cm cubic transparent enclosure that, on its lower horizontal surface, contained a 12-mm-diameter hole through which the rat's tongue extended down to reach the operandum (Fig. 1). The operandum was an 18 mm diameter aluminum disc rigidly attached to the shaft of a Model 31 load cell (Sensotec, Columbus, OH) and was centered 2 mm beneath the hole in the plastic enclosure. A computer-controlled peristaltic pump (Series E; Manostat Corp., New York, NY), fitted with a solid-state relay (Digikey, Thief River Falls, MN), and controlled by a LabMaster computer interface (LabMaster, Solon, OH), delivered water to the center of the lick disk through a 0.5-mm-diameter hole. The force transducer was capable of resolving force measurements to 0.2-g equivalent weights. A PC recorded the transducer's force-time output sampled at 100 samples/s.

2.3. Procedure

Following gradual water restriction, rats were exposed to the licking task during 6 minute sessions until they licked reliably at the 1-g force requirement (12 licks at or above criterion force were required to produce 0.05 ml of water). Each session started with a free 0.05 ml delivery of water. Upon stabilization of baseline, acute doses of GBR-12909 (5, 10, & 20 mg/kg) and then d-amphetamine (1 & 2 mg/kg) were administered subcutaneously, 30 minutes pre-session. Each rat received all doses of both drugs. Physiological saline was administered twice, once on each of the days immediately prior to the beginning of each drug's acute regimen. Doses were administered in ascending order of concentration (they were not counterbalanced), with the GBR-12909 regimen preceding amphetamine. Each drug dose was separated by at least two days. All rats completed the experiment.

2.2. Data analysis

The effects of age and drug dose on orolingual motor function were assessed in terms of three dependent variables: 1) number of licks per session, 2) the speed of licking in licks/second, and 3) the mean peak lick force. The number of licks was a count of the number of tongue contacts that equaled or exceeded 1 g. The licking speed was determined using the Fourier Spectral Analysis program in Autosignal (SeaSolve Software Inc., Framingham, MA). This method resolved lick rhythm to the nearest 0.1 Hz. This method was used because, unlike like rate, the peak in the power spectrum is not influenced by pauses in

licking behavior. The spectral analysis method is therefore more analogous to measures of within-burst inter-lick intervals (e.g., Davis & Smith, 1992). The peak lick force was the mean of the peak forces exerted during a session. Body weights were also analyzed as a function of age. Data for each measure were analyzed separately for each drug using a two-way analysis of variance (ANOVA) with age as the between-subjects variable and dose as the within-subjects repeating measure. The average of each drug's vehicle values was used as dose 0 for each drug. The level of significance was set at $p < 0.05$.

3. Results

At the time of testing, body weights were greater for the aged rats (490 ± 9 g) than for the young rats (290 ± 6 g); $F(1,18)=173.405$, $p < 0.0001$. Representative force-time waveforms illustrating licking bouts for one rat in each age group are presented in Figure 2. Unlike our previous studies, the number of licks per session did not differ between the two age groups (Fig. 3A). GBR-12909 increased this measure in the young, but not in the aged rats, leading to a significant drug X age group interaction, $F(3,54)=2.94$, $p < 0.05$. There was a significant main effect for amphetamine on number of licks, $F(2,36)=13.735$, $p < 0.001$, as it increased the measure. Post-hoc analyses revealed that this effect was significantly only for the 1 mg/kg dose ($p < 0.05$). As we have reported before, the aged group exhibited greater peak tongue force (Fig. 3B), leading to a significant main effect for age during the GBR-12909 regimen, $F(1,18)=7.465$, $p < 0.05$. GBR-12909 did not affect peak tongue force. The greater peak tongue force exhibited by the aged rats was decreased by amphetamine, leading to a significant main effect for drug, $F(2,34)=15.594$, $p < 0.001$, and a significant drug X age group interaction, $F(2,34)=5.717$, $p < 0.01$. Tongue motility (licks/second) was significantly lower in the aged group (Fig. 3C). This led to significant main effects for age during the GBR-12909, $F(1,18)=40.818$, $p < 0.001$, and the amphetamine, $F(1,18)=42.828$, $p < 0.001$ regimens. In addition, amphetamine significantly increased tongue motility, $F(2,36)=4.646$, $p < 0.05$. Post-hoc analyses revealed that this effect was significantly only for the 1 mg/kg dose ($p < 0.05$). GBR-12909 did not affect this measure.

4. Discussion

We report here differential effects of amphetamine and GBR-12909 on orolingual motor function in young vs aged F344/BN rats. Both drugs increased the number of licks/session, but GBR-12909 increased the measure only in the young group. Amphetamine, but not GBR-12909, decreased peak tongue force, primarily in the aged group. Amphetamine, but not GBR-12909, increased tongue motility in both groups.

Our finding that the older rats did not produce a greater number of licks/session stands in contrast to our previous work (Stanford et al., 2003; Zhang and Stanford, 2008; Zhang et al., 2008). We do not know what accounted for this lack of replication, but our numbers suggest the difference was greater motivation in the young group in the current study. The fact that both drugs increased this measure is consistent with previous studies reporting increased operant and spontaneous behavior following treatment with DA agonists, including GBR-12909 and amphetamine (Grilly and Loveland, 2001; Kelley and Lang, 1989; van den Buuse and de Jong, 1989). The fact that amphetamine's effects on this measure were limited

to the 1 mg/kg dose suggests that modest increases in locomotion may have interfered with task engagement. Although we did not measure locomotor activity, both amphetamine (Grilly and Loveland, 2001) and GBR-12909 (Kelley and Lang, 1989; van den Buuse and de Jong, 1989) increase locomotor activity in rats at the doses that we administered here. Age-related decreases in DA transporter number and function (Hebert and Gerhardt, 1999; Hebert et al., 1999) may have accounted for the lack of increase in number of licks in the older group with GBR-12909. In contrast, nomifensine was previously shown to increase locomotor activity (Stanford et al., 2002a; 2002b), but decrease the number of licks in both young and aged F344 rats (Stanford et al., 2003). While further studies are necessary to determine the effects of GBR-12909 on locomotor activity in aged rats, our current findings, along with those reviewed here, suggest complex relationships between aging, DA agonist mechanism of action, and type of motor behavior tested.

The reason for the dose-dependent decrease in peak tongue force in the aged rats following amphetamine is unclear. A previous study reported that amphetamine had no effect on tongue force in this task in young Sprague-Dawley rats (Fowler et al., 2005). There are substantial strain differences in this task, however, as Sprague-Dawley rats emit greater tongue forces than Fischer strains (F344 and F344/BN), even at the same force requirement (Fowler et al., 2005; Guggenmos et al., 2009; Nishimune et al., 2012; Smittkamp et al., 2010; Stanford et al., 2003; Zhang and Stanford, 2008; Zhang et al., 2008). We have reported differences between Fischer and Sprague-Dawley strains in regard to the time it takes animals to engage in this task (Stanford et al., 2003). Although speculative, affective differences may at least partly account for this phenomenon. Our finding that peak tongue force is elevated by lorazepam in both young and aged F344/BN rats (Zhang and Stanford, 2008) is consistent with this hypothesis. It is possible that amphetamine's documented anxiogenic effects (Pellow et al., 1985; Simon et al., 1993; Thiébot et al., 1991) contributed to this effect in this rat strain. An alternate explanation is that faster licking is accompanied by attenuated tongue protrusion. This would be consistent with the previous study regarding amphetamine's lack of effect on tongue force in young Sprague-Dawley rats, since licking speed was also not affected (Fowler et al., 2005).

Our studies have consistently reported an age-related decrease in tongue motility in rats (Stanford et al., 2003; Zhang and Stanford, 2008; Zhang et al., 2008). These findings are consistent with studies of elderly human subjects performing a repetitive tongue movement task (Hirai et al., 1991), thus increasing the validity of this task as a model of orolingual motility decline in aging humans. We have reported that licking speed in aged rats is positively correlated with measures of DA and DA turnover in the substantia nigra (Stanford et al., 2003). The age of onset of decreased tongue motility corresponds with the onset of changes in nigrostriatal function in F344/BN and F344 rats (Hebert et al., 1998; Yurek et al., 1998). Factors other than nigrostriatal DA may also play a role in age-related tongue motility deficits, however. For example, we recently reported that bilateral nigrostriatal DA depletion does not reduce tongue motility in young adult rats (Nuckolls, et al., 2012). Furthermore, our current results with GBR-12909, and our previous results with nomifensine (Stanford et al., 2003), suggest that increasing extracellular DA by blocking its reuptake does not improve tongue motility in aged rats. Normal aging is accompanied by decreases in DA receptors, with loss of the D2 type more widely reported than loss of D1

DA receptors (Stanford et al., 2001). Acute antagonism of D2 DA receptors with haloperidol have produced mixed results, with Fowler and Mortell (1992) reporting decreases and Fowler and Wang (1998) reporting no effects. The effects of selective D1 antagonists on tongue motility have not been reported.

Although the basal ganglia play a role in orolingual motor function, high speed licking behavior in rats is also influenced by central pattern generators in the hypoglossal nucleus, the cerebellum, and the brainstem reticular formation (Brozek et al., 1996; Welsh et al., 1995; Wiesenfeld et al., 1997). Age-related functional alterations have been reported for each of these regions (Behan and Brownfield, 1999; Gould et al., 1995; Hilber and Caston, 2001; Zhang et al., 1997). In addition to its effect on DA, amphetamine's effects in reversing transport (i.e., release) of NE and 5-HT may have played an important role in increasing tongue motility. Previous studies report that NE input to the hypoglossal nucleus is involved with orolingual motor-related central pattern generators (Aldes et al., 1992).

Electromyographic activity of the tongue protruder genioglossus muscle is increased by the 5-HT precursor 5-hydroxytryptophan (Berry & Hayward, 2003). On hypoglossal motor neurons, NE acts through the $\alpha 1$ adrenergic receptor (Parkis et al., 1995; Volgin et al., 2001), while 5-HT acts primarily on 5-HT_{2a} receptors (Zhan et al., 2002). The fact that $\alpha 1$ adrenergic and 5-HT_{2a/c} antagonists decrease licking speed in rats (Fowler et al., 2005) supports the role of these receptors in orolingual motor function. Supporting their role in age-related tongue motility deficits are findings that $\alpha 1$ adrenergic and 5-HT_{2a/c} receptors undergo loss or functional decline in aging (Baeken et al., 1998; Brunello et al., 1988; Druse et al., 1997; Meltzer et al., 1998; Zhou et al., 1984).

Despite the significant effect, the increase in tongue motility produced by amphetamine was modest. In fact, the effect was limited to the 1 mg/kg dose, which may indicate task disruption at the higher dose. Further studies examining interventions that increase presynaptic DA release and movement speed in aging are clearly warranted given our results. Strategies that increase glial cell line-derived neurotrophic factor (GDNF; Hebert & Gerhardt, 1997), neurturin (Cass & Peters, 2010), or GFR $\alpha 1$ /GFR $\alpha 2$ (receptors for GDNF and neurturin, respectively; Pruett & Salvatore, 2013) are promising. Interventions that increase signaling at the 5-HT_{2a} and $\alpha 1$ adrenergic receptors have not been tested in aged animals and are also promising candidates. Although normal aging is not associated with loss of hypoglossal motor neurons (Sturrock, 1991), contractile properties of tongue muscles are affected by aging (Ota et al., 2005). Physiotherapeutic interventions such as tongue strength training can increase tongue force and neuromuscular function in aged animals (Behan et al., 2012; Connor et al., 2009; Guggenmos et al., 2009; Nishimune et al., 2012; Schaser et al., 2012). While tongue strength training has not been shown to improve age-related tongue motility deficits, further studies are necessary to determine whether it might enhance the effects of pharmacological interventions.

Acknowledgments

This work was supported by NIH grants AG023549, AG026491, a KUMC Biomedical Research Training grant and the KIDDRC Center grant HD02528.

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Figure 1.

Image of a rat making an individual tongue contact with the lick disc. The tongue extends through a 12-mm diameter hole in the bottom of the transparent enclosure. On the left is the tubing through which water is pumped through a hole drilled into the side of the aluminum disc. The hole is drilled such that the water enters the side of the disc and exits the top through a hole at the center. The plastic splash cone protects the transducer housing (large cylinder) from water.

Figure 2.

Raw force-time waveforms selected from a representative (A) young adult (6-month-old) and (B) aged (30-month-old) F344/BN rat. Time (in seconds) runs from the left to the right of each abscissa (i.e., two lines depict 20 seconds of a 6 minute session). The greater peak force (11.4 vs 7.1 g) and slower licking speed (4.9 vs 5.5 licks/s) are apparent in the older rat.

Figure 3.

Orolingual motor measures as a function of age and drug dose. (A) Number of licks per session was increased by GBR-12909, but only in the young rats ($^x p < 0.05$). Unlike GBR-12909, amphetamine increased number of licks per session in both groups ($^* p < 0.001$). Post hoc analysis revealed that this effect was significant only at 1 mg/kg ($^p p < 0.05$). (B) Peak tongue force was greater in the old group ($^# p < 0.05$), and was not affected by GBR-12909. Amphetamine decreased peak force, but only in the older rats ($^x p < 0.01$). (C) Licking speed was slower in the older group ($^# p < 0.001$). This measure was increased by amphetamine ($^* p < 0.05$). Post hoc analysis revealed that this effect was significant only at 1 mg/kg ($^p p < 0.05$). Explanation of symbols: * main effect for drug; $^#$ main effect for age; x drug-by-age interaction; p significant post-hoc comparison.