

Increased cholinergic drive in the CNS contributes to apneas in active sleep displayed

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by infant rats deficient in central serotonin

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Abstract:

Sudden Infant Death Syndrome (SIDS), the most common cause of death in infants between 1 month and 1 year of age, occurs during sleep. There is evidence that SIDS cases have sleep apnea and prolonged periods of active sleep (AS; similar to rapid eye movement sleep in adults) prior to death. Apnea and the resulting hypoxia are likely key factors in the sudden death of these infants during sleep. Studies have shown SIDS cases have abnormalities in the serotonin (5-hydroxytryptamine; 5-HT) neurons in the brainstem, including reduced 5-HT in the CNS. Infant rodents lacking 5-HT experience apnea during prolonged episodes of AS. Given that 1) acetylcholine is a major driver of AS; 2) AS-driving, cholinergic neurons project widely to brainstem neurons that control respiratory patterning; and 3) 5-HT can inhibit AS-driving cholinergic neurons, we hypothesize that increased cholinergic drive within the brainstem contributes to the unstable breathing pattern of 5-HT-deficient rat pups. To test this hypothesis, we are using 14-16-day-old rat pups lacking tryptophan hydroxylase 2 (TPH2^{-/-}), the enzyme catalyzing the rate-limiting step of 5-HT biosynthesis within the CNS, as well as wild-type controls. For both genotypes, we have three groups: 1) pups treated systemically with atropine sulfate (1 mg/kg; *i.p.*), which is CNS permeable, as the experimental group (n=9); 2) pups treated with vehicle (saline) as a control (n=8); 3) pups treated with atropine methyl nitrite, which is not CNS permeable, to control for the effects of atropine on the heart (n=8). Quiet sleep (QS; similar to non-REM sleep in adults) and AS were determined using nuchal electromyography (EMG) and behavioral observation. Whole-body plethysmography was used to monitor the breathing pattern while the pups cycled between AS and QS before and after drug administration. TPH2^{-/-} pups tend to have fewer apneas following atropine treatment (p=0.07 compared to vehicle control). Experiments on all groups, including wildtype pups, are on-going. These results help support our hypothesis that increased cholinergic drive during AS contributes to the apneas experienced by future SIDS cases.

Introduction

- Sudden Infant Death Syndrome (SIDS) occurs during sleep and is the most common cause of death in infants between the ages of 1 month-1 year of age
- There is evidence that SIDS cases have sleep apnea prior to death
- Apnea and resulting hypoxia are likely key factors in the sudden death of these infants during sleep
- Studies have show SIDS cases have abnormalities in the serotonin (5-hydroxytryptamine; 5-HT) neurons in the brainstem, including reduced 5-HT
- Infant rats deficient in 5-HT experience irregular breathing and apnea during prolonged periods of active sleep (AS; similar to REM in adults)
- It may be that enhanced cholinergic drive contributes to the apneas demonstrated by 5-HT deficient pups, given that: 1) acetylcholine is a major driver of AS; 2) AS-driving cholinergic neurons project widely to respiratory patterning neurons; and 3) 5-HT inhibits cholinergic neurons

Hypothesis

We hypothesize that increased cholinergic drive contributes to the unstable breathing pattern of 5-HT-deficient rat pups during prolonged periods of AS

Methods

Animals and Groups:

We will use 14-16-day-old rat pups lacking tryptophan hydroxylase 2 (TPH2^{-/-}), the enzyme catalyzing the rate-limiting step of 5-HT biosynthesis, as well as wild-type (WT) controls. For both genotypes, we will have three groups:

Group 1: pups treated with atropine sulfate (CNS permeable) as the experimental group (n=9 TPH2^{-/-}, to date)

Group 2: pups treated with saline to control for the injection (n=8 TPH2^{-/-}, to date)

Group 3: pups treated with atropine methyl nitrate (non-CNS permeable) to control for the effects of atropine on the heart (none tested to date)

Surgery:

We resolved wakefulness, active sleep (AS; similar to REM sleep in adults) and quiet sleep (QS; similar to non-REM sleep in adults) using nuchal electromyography. Under ~2% isoflurane, two electrodes were implanted: one in the nuchal muscles and another in the back musculature to act as a ground.

Experimental Protocol:

Following surgery, pups were given a 20-30 min settling period in a warmed (31°C) plethysmography chamber with constant (300mL/min) flow of air (Fig.1a). Pups were allowed to cycle between QS and AS for a maximum of 2 hours prior to saline or atropine injection (1 mg/kg; *i.p.*). Following drug injection, we recorded sleep and breathing for an additional 2 hours.

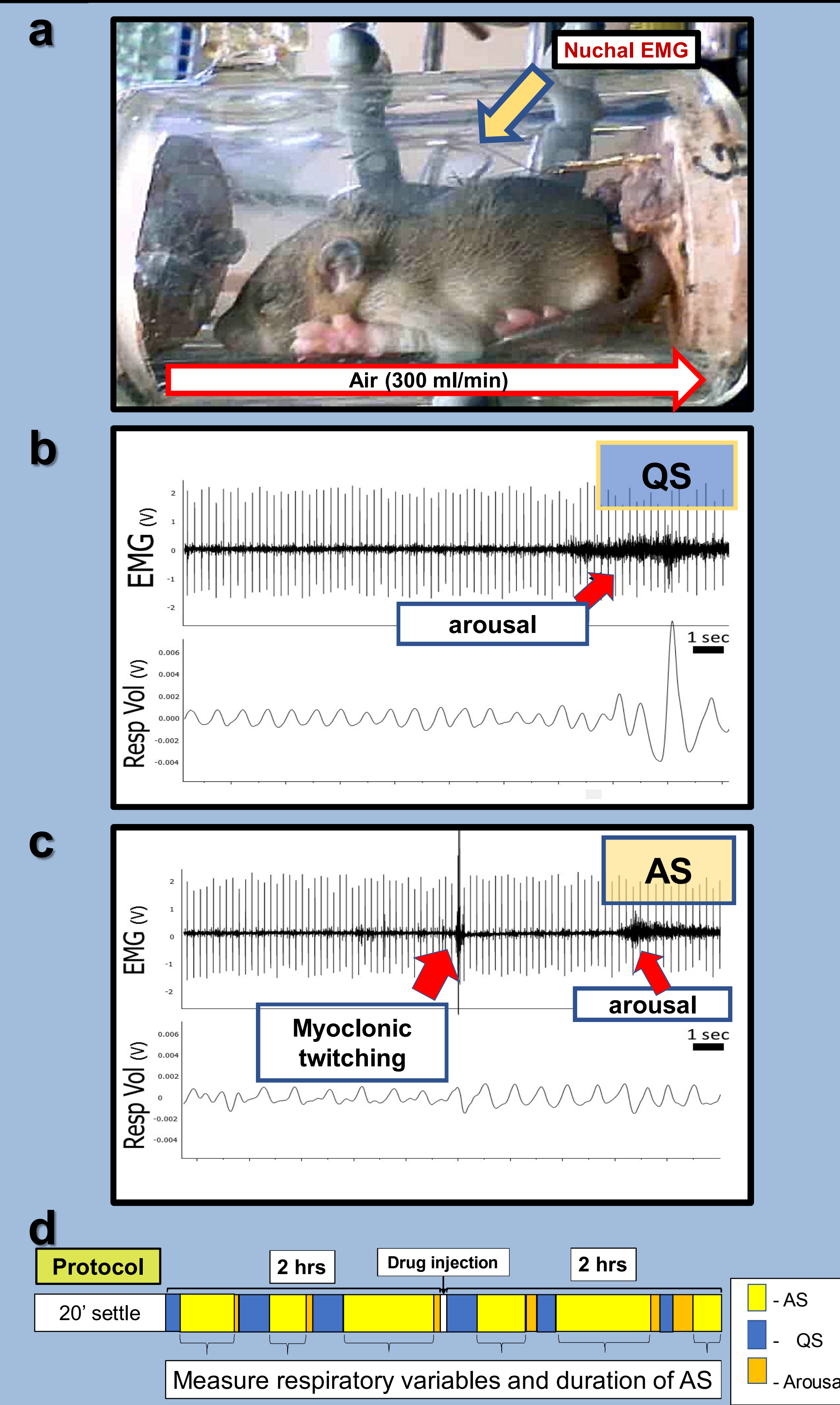
Measurements:

Sleep state was determined using nuchal EMG and behavioral observation (n=15) or behavioral observation alone (n=2). Breathing was monitored with whole-body plethysmography. Frequency of breathing (f_B) and tidal volume (V_T) were measured. HR was determined using the electrocardiographic activity detected on the EMG records. Respiratory period (P) was used to detect and quantify apneas and tachypneas. Apneas and tachypneas were defined as any P that was at least 1.5 times longer (apnea) or shorter (tachypnea) than the average P for that animal prior to drug or saline injection. The frequency of apnea and tachypneas were expressed as apnea and tachypnea indices (number of events/hr).

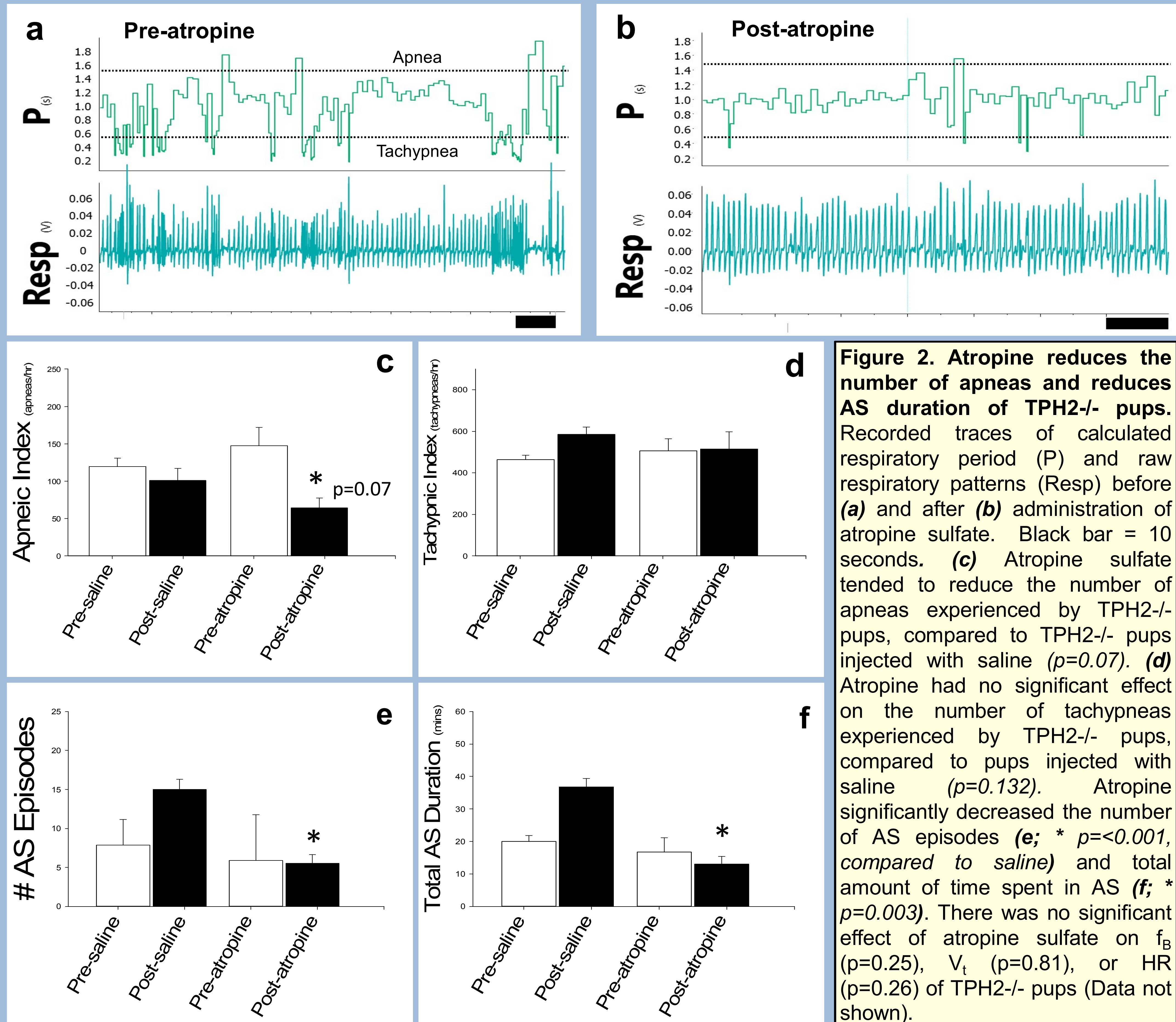
Note: we present variables from AS only.

Data and Statistical Analyses:

We used two-way repeated measures analysis of variance to assess significant effects of atropine sulfate compared to saline injection on respiratory variables, including the number of apneas/hr. Significant effects were determined at p<0.05



Results



Conclusions

- Compared to TPH2^{-/-} pups injected with saline, pups injected with atropine experienced fewer apneas during AS. More animals will be tested to establish whether or not this effect is statistically significant.
- Atropine sulfate had no significant influence on the number of tachypneas experienced by TPH2^{-/-} pups during AS.
- Atropine sulfate significantly decreased the number of AS episodes, and overall amount of time TPH2^{-/-} pups spent in AS.
- These data suggest that increased cholinergic drive within the CNS contributes to the increased apneas, but not tachypneas, experienced by infant rats deficient in 5-HT.

Future Directions

- Atropine sulfate has significant effects on cardiovascular function, including reducing heart rate variability. To control for any of these effects, we will also test the effects of atropine methyl nitrite, which is incapable of crossing the blood brain barrier, on the apneas of TPH2^{-/-} pups. We hypothesize that this drug will not reduce the apneas of TPH2^{-/-} pups.
- We will test the effects of atropine sulfate and methyl nitrite on the breathing pattern of WT animals. Our hypothesis is that atropine sulfate has a larger effect in TPH2^{-/-} pups than WT, reflecting the increased cholinergic drive in TPH2^{-/-} compared to WT.
- To resolve the location in which central 5-HT helps to reduce cholinergic drive and mitigate apnea, we will microinject atropine sulfate into specific brainstem respiratory centers (eg. in the pons, which contains groups of neurons that terminate inspiration; *i.e.* promote apnea).